

#### (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

# (19) World Intellectual Property Organization International Bureau





(43) International Publication Date 22 December 2005 (22.12.2005)

**PCT** 

# (10) International Publication Number WO 2005/121364 A2

(51) International Patent Classification<sup>7</sup>:

C12Q 1/68

(74) Agent: G.E. EHRLICH (1995) LTD.; 11 Menachem Begin Street, 52 521 Ramat Gan (IL).

(21) International Application Number:

PCT/IL2005/000627

(22) International Filing Date: 14 June 2005 (14.06.2005)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/578,833

14 June 2004 (14.06.2004) US

- (71) Applicant (for all designated States except US): EVO-GENE LTD. [IL/IL]; 13 Gad Finstein Street, 76121 Rehovot (IL).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): RONEN, Gil [IL/IL]; 20 Moshav Ometz, 33870 Emeq-Hefer (IL). GOLD, Evgenia [IL/IL]; 11/3 Yavne Street, 76360 Rechovot (IL). YELIN, Rodrigo [IL/IL]; 12 Odem Street, 44862 Zur-Yigal (IL). MEISSNER, Rafael [IL/IL]; 11 HaLevona Street, 76350 Rechovot (IL). KARCHI, Hagai [IL/IL]; 249 Sapir Street, Moshav Sitriya, 76834 Doar-Na Emek Soreq (IL).

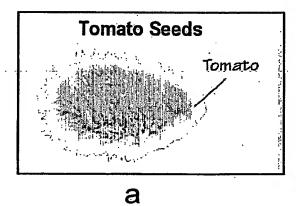
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
  - GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

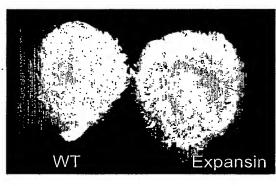
#### Published:

 without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYNUCLEOTIDES AND POLYPEPTIDES INVOLVED IN PLANT FIBER DEVELOPMENT AND METHODS OF USING SAME





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(57) Abstract: Isolated polynucleotides are provided. Each of the isolated polynucleotides comprise a nucleic acid sequence encoding a polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96, wherein the polypeptide is capable of regulating cotton fiber development. Also provided are methods of using such polynucleotides for improving fiber quality and/or yield of a fiber producing plant, as well as methods of using such polynucleotides for producing plants having increased biomass/vigor/yield.





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# POLYNUCLEOTIDES AND POLYPEPTIDES INVOLVED IN PLANT FIBER DEVELOPMENT AND METHODS OF USING SAME

# FIELD AND BACKGROUND OF THE INVENTION

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The present invention relates to polynucleotides and polypeptides involved in plant-fiber development and methods of using same.

The present invention relates to a novel computational approach that utilizes comparative genomics to identify genes which play a role in fiber development.

Cotton and cotton by-products provide raw materials that are used to produce a wealth of consumer-based products in addition to textiles including cotton foodstuffs, livestock feed, fertilizer and paper. The production, marketing, consumption and trade of cotton-based products generate an excess of \$100 billion annually in the U.S. alone, making cotton the number one value-added crop.

It is estimated that the use of cotton as a fiber by humans dates back 7000 years in Central America and 5000 years in India. Even with the growth of synthetic fibers in the last 50 years, cotton still accounts for approximately 50 % of the world's textile fiber [Agrow Reports, Global Seed markets DS208, October 2000].

Even though 90 % of cotton's value as a crop resides in the fiber (lint), yield and fiber quality has declined, especially over the last decade [Meredith (2000), Proc. World Cotton Research Conference II, Athens, Greece pp.97-101]. This decline has been attributed to general erosion in genetic diversity of cotton varieties, and an increased vulnerability of the crop to environmental conditions [Bowman et al., Crop Sci. 36:577-581 (1996); Meredith, supra].

There are many varieties of cotton plant, from which cotton fibers with a range of characteristics can be obtained and used for various applications. Cotton fibers may be characterized according to a variety of properties, some of which are considered highly desirable within the textile industry for the production of increasingly high quality products and optimal exploitation of modem spinning technologies. Commercially desirable properties include length, length uniformity, fineness, maturity ratio, decreased fuzz fiber production, micronaire, bundle strength, and single fiber strength. Much effort has been put into the improvement of the characteristics of cotton fibers mainly focusing on fiber length and fiber fineness. In particular, there is a great demand for cotton fibers of specific lengths.

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Methods for improving the characteristics or yield of cotton fibers can be classified into the following three categories:

### 1. Variety improvement by cross breeding

This method has been utilized most widely so far. At present, almost all the cultivated varieties of cotton plant are bred by this method. However, improvement of cotton fiber yield using traditional breeding is relatively slow and inefficientand the degree of variability which can be achieved is limited.

## 2. Treatment with plant hormones

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Plant hormones such as auxin, gibberellin, cytokinin and ethylene have been widely used in field crops or horticultural products. The influence of plant hormones, particularly gibberellin, auxin and brassinolide, on the fiber characteristics of cotton plants is known [e.g. U.S. Pat. No. 5880110 produces cotton fibers with improved fiber characteristics by treatment with brassinosteroids]. However, no measurable effect has been documented, making practical use of these hormones on a large scale highly unlikely.

### 3. Variety improvement by genetic engineering:

The broad acceptance of genetically engineered cotton in the leading producing countries and the fact that it is a non-food crop make it an attractive candidate for genetic engineering for improvement of fiber yield and/or quality.

In recent years, remarkable progress has been made in plant genetic engineering, as a result several cases of successful variety improvement of commercially important crop plants have been reported (e.g., cotton, soybean, corn, canola, tomato). For example, methods of improving insect resistance by the introduction of a gene coding for BT toxin (i.e., insecticidal protein toxin produced by Bacillus thuringiensis) in a cotton plant, have been developed and put to practical use. In addition, cotton plants with improved herbicide (Glyphosate) resistance have been genetically engineered by the introduction of a gene coding for 5-enol-pyruvil-shikimic acid 3-phosphate synthetase.

The availability and success of plant genetic engineering combined with the fact that cotton is an excellent candidate for genetic manipulation via recombinant techniques have led researchers to postulate that if a gene associated with an improved cotton fiber property could be identified, it could be up-regulated using recombinant techniques thus improving the characteristics or yield of cotton fibers.

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Conversely, if a gene associated with a decline in a cotton fiber property could be identified, it could be down-regulated using gene silencing methods. For this purpose, the mechanisms of fiber elongation and formation must be elucidated on the genetic level and genes closely associated with these mechanisms must be identified.

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A cotton fiber is composed of a single cell that has differentiated from an epidermal cell of the seed coat, developing through four stages, i.e., initiation, elongation, secondary cell wall thickening and maturation stages. More specifically, the elongation of a cotton fiber commences in the epidermal cell of the ovule immediately following flowering, after which the cotton fiber rapidly elongates for approximately 21 days. Fiber elongation is then terminated, and a secondary cell wall is formed and grown through maturation to become a mature cotton fiber.

Several candidate genes have been isolated which are associated with the elongation and formation of cotton fibers. For example, five genes from cotton plants have been identified that are specifically expressed at the cotton fiber elongation stage by differential screening method and differential display method, [U.S. Pat. No. 5,880,100 and U.S. patent applications Ser. Nos. 08/580,545, 08/867,484 and 09/262,653].

WO0245485 describes methods and means to modulate fiber quality in fiber-producing plants, such as cotton, by modulating sucrose synthase (a sugar important for cell wall synthesis) activity and/or expression in such plants.

U.S. Pat. No. 6,472,588 and WO0117333 provide methods for increasing the quality of cotton fiber produced from a cotton plant by transformation with a DNA encoding sucrose phosphate synthase. The fiber qualities include strength, length, fiber maturity ratio, immature fiber content, fiber uniformity and micronaire.

WO9508914 discloses a fiber producing plant comprising in its genome a heterologous genetic construct. The genetic construct comprises a fiber-specific promoter and a coding sequence encoding a plant peroxidase, such as a cotton peroxidase.

WO9626639 provides methods whereby an ovary specific promoter sequence is utilized to express plant growth modifying hormones in cotton ovule tissue. The methods permit the modification of the characteristics of boll set in cotton plants and provide a mechanism for altering fiber quality characteristics such as fiber dimension and strength.

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U.S. Pat. No. 5,981,834, U.S. Pat. No. 5,597,718, U.S. Pat. No. 5,620,882, U.S. Pat. No. 5,521,708 and U.S. Pat. No. 5,495,070 all disclose a method for genetically engineering a fiber-producing plant and the identification of cDNA clones useful for identifying fiber genes in cotton. The cDNA clones are useful in developing corresponding genomic clones from fiber producing plants to enable genetic engineering of cotton and other plants using these genes. Coding sequences from these isolated genes are used in sense or antisense orientation to alter the fiber characteristics of transgenic fiber producing plants.

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U.S. patent applications U.S. 2002049999 and U.S. 2003074697 both disclose cotton plants of the genus Gossypium with improved cotton fiber characteristics. The cotton plant has an expression cassette containing a gene coding for an enzyme selected from the group consisting of endoxyloglucan transferase, catalase and peroxidase so that the gene is expressed in cotton fiber cells to improve the cotton fiber characteristics.

WO 01/40250 provides methods for improving cotton fiber quality by modulating transcription factor gene expression.

WO 96/40924 provides novel DNA constructs which may be used as molecular probes or alternatively inserted into a plant host to provide for modification of transcription of a DNA sequence of interest during various stages of cotton fiber development. The DNA constructs comprise a cotton fiber transcriptional initiation regulatory region associated with a gene, which is expressed in cotton fiber. Also provided is a novel cotton having a cotton fiber which has a natural color. The color was achieved by the introduction and expression in cotton fiber cell of a pigment gene construct.

EP0834566 provides a gene which controls the fiber formation mechanism in cotton plant and which can be used for industrially useful improvement.

However, beside Sucrose Synthase, there is no evidence to date that the expression of any particular gene plays an essential role in cotton fiber formation or enhanced fiber characteristics.

Thus, there remains a need for identifying other genes associated with fiber characteristics of cotton plants and a more thorough search for quality-related genes is required.

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While reducing the present invention to practice the present inventors devised and employed a novel computational approach that utilizes comparative genomics to identify genes which play a pivotal role in fiber development. As demonstrated herein, expression of such genes correlates with fiber length and their overexpression is sufficient to modify tomato seed hair, an ultimate model for cotton fibers. These results suggest that polynucleotides of the present invention can be used for generating transgenic cotton plants which are characterized by fibers of desired length.

#### 10 SUMMARY OF THE INVENTION

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According to one aspect of the present invention there is provided an isolated polynucleotide comprising a nucleic acid sequence encoding a polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96, wherein the polypeptide is capable of regulating cotton fiber development.

According to further features in preferred embodiments of the invention described below, the nucleic acid sequence is selected from the group consisting of SEQ ID NOs. 1, 2, 4, 5, 7, 9, 10, 16, 17, 20, 21, 22, 24, 25, 27 and 13.

According to still further features in the described preferred embodiments the polypeptide is as set forth in SEQ ID NO. 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96.

According to still further features in the described preferred embodiments the amino acid sequence is as set forth in SEQ ID NO. 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96.

According to still further features in the described preferred embodiments the cotton fiber development comprises fiber formation.

According to still further features in the described preferred embodiments the cotton fiber development comprises fiber elongation.

According to another aspect of the present invention there is provided an isolated polynucleotide comprising a nucleic acid sequence at least 80 % identical to SEQ ID NO: 85 or 91, wherein the nucleic acid sequence is capable of regulating expression of at least one polynucleotide sequence operably linked thereto in an ovule endothelial cell.

According to still further features in the described preferred embodiments the ovule endothelial cell is of a plant fiber or a trichome.

According to yet another aspect of the present invention there is provided an oligonucleotide capable of specifically hybridizing to the isolated polynucleotide.

According to another aspect of the present invention there is provided a nucleic acid construct comprising the isolated polynucleotide.

According to still further features in the described preferred embodiments the nucleic acid construct further comprising at least one cis-acting regulatory element operably linked to the isolated polynucleotide.

According to still further features in the described preferred embodiments the polynucleotide sequence is selected from the group consisting of SEQ ID NOs: 1, 2, 4, 5, 7, 9, 10, 16, 17, 20, 21, 22, 24, 25, 27 and 13.

According to still further features in the described preferred embodiments the cis-acting regulatory element is as set forth in SEQ ID NO: 74, 75, 85 or 91 or functional equivalents thereof.

According to an additional aspect of the present invention there is provided a transgenic cell comprising the nucleic acid construct.

According to yet an additional aspect of the present invention there is provided a transgenic plant comprising the nucleic acid construct.

According to yet another aspect of the present invention there is provided a method of improving fiber quality and/or yield of a fiber producing plant, the method comprising regulating an expression level or activity of at least one polynucleotide encoding a polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96 in the fiber producing plant, thereby improving the quality and/or yield of the fiber producing plant.

According to still further features in the described preferred embodiments the quality of the fiber producing plant comprises at least one parameter selected from the group consisting of fiber length, fiber strength, fiber weight per unit length, maturity ratio, uniformity and micronaire.

According to still further features in the described preferred embodiments the regulating expression or activity of the at least one polynucleotide is up-regulating.

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According to still further features in the described preferred embodiments the up-regulating is effected by introducing into the cotton the nucleic acid construct.

According to still further features in the described preferred embodiments the regulating expression or activity of the at least one polynucleotide is down-regulating.

According to still further features in the described preferred embodiments the down-regulating is effected by gene silencing.

According to still further features in the described preferred embodiments the gene silencing is effected by introducing into the cotton the oligonucleotide.

According to still further features in the described preferred embodiments the fiber producing plant is selected from the group consisting of cotton, silk cotton tree (Kapok, Ceiba pentandra), desert willow, creosote bush, winterfat, balsa, ramie, kenaf, hemp, roselle, jute, sisal abaca and flax.

According to still an additional aspect of the present invention there is provided a method of increasing a biomass of a plant, the method comprising regulating an expression level or activity of at least one polynucleotide encoding a polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96 in the plant, thereby increasing the biomass of the plant.

According to still further features in the described preferred embodiments the plant is a monocot plant.

According to still further features in the described preferred embodiments the plant is a dicot plant.

According to a further aspect of the present invention there is provided a method of identifying genes which are involved in cotton fiber development, the method comprising:

- (a) providing expressed nucleic acid sequences derived from cotton fibers;
- (b) providing expressed nucleic acid sequences derived from an ovule tissue;
- (c) computationally assembling the expressed nucleic acid sequences of
  (a) and (b) to generate clusters; and
- (d) identifying clusters of the clusters which comprise expressed nucleic acid sequences of (a) and (b), thereby identifying genes which are involved in cotton fiber development.

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According to still further features in the described preferred embodiments the method further comprising identifying genes which are differentially expressed in the cotton fiber following (d).

According to still further features in the described preferred embodiments the differentially expressed comprises:

- (a) specific expression; and/or
- (b) change in expression over fiber development.

According to yet an additional aspect of the present invention there is provided a method of producing an insect resistant plant, comprising regulating an expression level or activity of at least one polynucleotide encoding a polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96 in a trichome of the plant, thereby producing the insect resistant plant.

According to still an additional aspect of the present invention there is provided a method of producing cotton fibers, the method comprising:

- (a) generating a transgenic cotton plant expressing at least one polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96; and
- (b) harvesting the fibers of the transgenic cotton plant, thereby producing the cotton fibers.

The present invention successfully addresses the shortcomings of the presently known configurations by providing genes involved in cotton fiber development and methods of using same.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

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### BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

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FIG. 1 is an illustration depicting the bioinformatic methodology of the present invention effected to identify genes which may be used to improve cotton fiber yield and quality.

FIGs. 2a-d are bar graphs showing expression patterns of fiber specific genes (CT\_11 Figure 2b), elongation associated genes (CT\_1, Figure 2c) and initiation associated genes (CT\_22, Figure 2d).

FIG. 3 is a graph depicting expression of CT\_76 in varieties of cotton (G. hirsutum var Tamcot, Coker and Acala, and G. barbadense var Pima S5) plants, as determined by RT-PCR.

FIG. 4 is a schematic illustration of the pPi binary plasmid.

FIGs. 5a-l are photographs of wild-type and transgenic arabidopsis plants over-expressing genes of the present invention. Figure 5a shows two week old rosette of wt plants; Figure 5b shows two week old rosette of CT11 over-expressing arabidopsis plants; Figure 5c shows two week old roots of CT11; Figure 5d shows three week old wild type arabidopsis; Figure 5e shows three week old CT\_20; Figure 5f shows three week old CT\_22; Figure 5g shows 30 days old rosettes of wt and CT\_9; Figure 5h shows 30 days inflorescence of wt and CT\_9; Figure 5i shows two week old roots of CT9; Figure 5j shows 30 days old rosettes of wt and CT\_40; Figure 5k shows rosette of 5 week old wt and CT81 over-expressing plants; Figure 5l shows a leaf of wt and CT81 over-expressing arabidopsis plants;

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FIGs. 6a-e are photographs depicting wild-type and transgenic tomato plants over-expressing CT\_20. Figure 6a shows a leaf of wild-type plant; Figure 6b shows a leaf of CT\_20 transgenic tomato; Figure 6c shows seed hairs of WT and CT\_20 over-expressing tomato plants; Figure 6d shows section of a wt tomato seed; Figure 6e shows section of a CT\_20 over-expressing tomato seed; Figure 6f seed hairs of WT and CT\_82.

FIGs. 7a-b are photographs depicting transgenic tomato plants over-expressing GUS under the expression of the CT\_2 promoter. Figure 7a is a cut through transgenic tomato fruit, over-expressing GUS under CT2 promoter in the mature green stage (x 5 magnification). Figure 7b similar to Figure 7a showing x 25 magnification;

FIGs. 8a-b are photographs depicting various magnifications of wild-type and transgenic tomato fruits or tomato seeds. Figure 8a is a single wild type tomato seed covered with seed hairs x 10 magnification; Figure 8b shows tomato seed over expressing expansin under 35S (x 10 magnification).

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# DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of polypeptides and polynucleotides encoding same which are involved in plant fiber development and which can be used to improve fiber quality and/or yield/biomass of a fiber producing plant.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Cotton and cotton by-products provide raw materials that are used to produce a wealth of consumer-based products; in addition to textiles, cotton is used to produce foodstuffs, livestock feed, fertilizer and paper. The production, marketing, consumption and trade of cotton-based products generate an excess of \$100 billion annually in the U.S. alone, making cotton the number one value-added crop.

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Over the past decade cotton fiber production has sharply declined prompting cotton growers and researchers to look for approaches, which can be used to improve fiber yield and quality.

Increasing fiber quality and/or yield under diverse environmental conditions will increase the profitability of cotton crop production and provide a new spectrum of material properties for exploitation by the processing industries.

While reducing the present invention to practice, the present inventors have configured a novel computational approach that utilizes comparative genomics to identify genes which play a role in fiber development. Genes identified using this approach may be successfully used for generating transgenic plants which are featured by fibers of desired properties.

Thus, according to one aspect of the present invention there is provided a method of identifying genes which are involved in cotton fiber development.

As used herein the term "cotton" refers to a wild-type, a cultivated variety (e.g., hybrid) or a transgenic cotton (Gossypium) plant.

As used herein the phrase "fiber development" refers to the development of the hair of the cotton seed.

As used herein the term "development" when used in context of cotton fibers refers to initiation of the fiber and/or elongation thereof, as well as to the fiber secondary cell wall thickening and maturation.

The method according to this aspect of the present invention is effected by:

- (a) providing expressed nucleic acid sequences derived from cotton fibers;
- (b) providing expressed nucleic acid sequences derived from an ovule tissue (i.e., a tissue developed from an ovary of a seed plant. Examples include, but are not limited to, carpels, seed coat, embryo, endosperm);
- (c) computationally assembling the expressed nucleic acid sequences of (a) and (b) to generate clusters; and
- (d) identifying clusters of said clusters which comprise expressed nucleic acid sequences of (a) and (b), thereby identifying genes which are involved in cotton fiber development.

Expressed nucleic acid sequences used as a potential source for identifying genes involved in cotton fiber development according to this aspect of the present invention are preferably libraries of expressed messenger RNA [i.e., expressed

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sequence tags (EST), cDNA clones, contigs, pre-mRNA, etc.] obtained from tissue or cell-line preparations which can include genomic and/or cDNA sequence.

Expressed nucleic acid sequences, according to this aspect of the present invention can be retrieved from pre-existing publicly available databases (see Example 1 of the Examples section which follows or private databases).

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Alternatively, the expressed nucleic acid sequences utilized by the present invention can be generated from sequence libraries (e.g., cDNA libraries, EST libraries, mRNA libraries and others).

cDNA libraries are suitable sources for expressed sequence information.

Generating a sequence database in such a case is typically effected by tissue or cell sample preparation, RNA isolation, cDNA library construction and sequencing.

It will be appreciated that such cDNA libraries can be constructed from RNA isolated from whole plant, specific tissues, or cell populations.

Once expressed sequence data is obtained from both cotton fibers and an ovule tissue, sequences may be clustered to form contigs. See Example 1 of the Examples section which follows

Such contigs are then assembled to identify homologous sequences (of cotton fibers and ovule tissue) present in the same cluster, such contigs are considered to be involved in cotton fiber development.

A number of commonly used computer software fragment read assemblers capable of forming clusters of expressed sequences are commercially available. These packages include but are not limited to, The TIGR Assembler [Sutton G. et al. (1995) Genome Science and Technology 1:9-19], GAP [Bonfield JK. et al. (1995) Nucleic Acids Res. 23:4992-4999], CAP2 [Huang X. et al. (1996) Genomics 33:21-31], The Genome Construction Manager [Laurence CB. Et al. (1994) Genomics 23:192-201], Bio Image Sequence Assembly Manager, SeqMan [Swindell SR. and Plasterer JN. (1997) Methods Mol. Biol. 70:75-89], LEADS and GenCarta (Compugen Ltd. Israel).

Once genes which are involved in cotton fiber development are identified their pattern of expression can be analyzed as described in Example 2 of the Examples section which follows, to thereby identify genes which are differentially expressed in the cotton fiber (i.e., specific expression) or during cotton fiber development (i.e., change in expression during cotton fiber development).

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Methods of identifying differentially expressed genes are well known in the art.

Using the above methodology, the present inventors were able to successfully identify genes which are involved in cotton fiber development.

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As is illustrated in the Examples section which follows genes identified using the teachings of the present invention can be classified into 6 functional categories according to their sequence homology to known proteins and enzymes (Table 3, below). The Two genes were classified into a cell fate commitment category: homologous to the MYB transcription factor and to GL3 which are known to be involved in trichome development in arabidopsis. The expression pattern of both genes and the phenotype of CT20 transgene both in arabidopsis and tomato T1 plants support their involvement mainly in the initiation phase. Two other genes (Table 3, above) are transcription factors from the MYB and MADS BOX families. Many studies demonstrated the function of these two transcription factor families as homeotic genes with key role in different developmental processes, among them are trichome and fiber morphogenesis (Suo. J. et. al. 2003, Ferrario S et. al. 2004). Their role in early stages of fiber development is supported also by their RNA expression pattern, which, is induced before, and during the day of anthesis. One gene belongs to the pathways of starch and sucrose metabolism. A recent work demonstrates that another gene (SUS), which, belongs to this pathway, is a limiting factor in both fiber initiation and development. Another gene (Table 3, below) is classified as lipid transport whose RNA expression is highly induced during early fiber elongation stage fit to the fact that lipids are key components in fiber formation. Several genes (Table 3, below) were classified either as genes involved in desiccation, salinity response stimulated by abscisic acid and genes involved in electron transfer. Out of them 3 genes were selected by RNA expression pattern to be induced in the elongation stage.

In view of the above and together with the experimental results which correlate gene expression with fiber length, it is suggested that genes of the present invention can be used to generate fiber producing plants with commercially desired fiber quality.

Thus, the present invention encompasses polynucleotides identified using the present methodology and their encoded polypeptide as well as functional equivalents of the polypeptides identified herein (i.e., , polypeptides which are capable of

regulating cotton fiber development, as can be determined according to the assays described in the Examples section which follows) and their coding sequences. Such functional equivalents can be at least about 70 %, at least about 75 %, at least about 80 %, at least about 81 %, at least about 82 %, at least about 83 %, at least about 84 %, at least about 85 %, at least about 86 %, at least about 87 %, at least about 88 %, at least about 89 %, at least about 90 %, at least about 91 %, at least about 92 %, at least about 93 %, at least about 94 %, at least about 95 %, at least about 75 %, at l

Polynucleotides encoding functional equivalents can be at least about 70 %, at least about 75 %, at least about 80 %, at least about 81 %, at least about 82 %, at least about 83 %, at least about 84 %, at least about 85 %, at least about 86 %, at least about 87 %, at least about 88 %, at least about 89 %, at least about 90 %, at least about 91 %, at least about 92 %, at least about 93 %, at least about 94 %, at least about 95 %, at least about 75 %, at least about 75

Homology (e.g., percent homology) can be determined using any homology comparison software, including for example, the BlastP software of the National Center of Biotechnology Information (NCBI) such as by using default parameters.

Identity (e.g., percent homology) can be determined using any homology comparison software, including for example, the BlastN software of the National Center of Biotechnology Information (NCBI) such as by using default parameters.

As used herein the phrase "an isolated polynucleotide" refers to a single or double stranded nucleic acid sequences which is isolated and provided in the form of an RNA sequence, a complementary polynucleotide sequence (cDNA), a genomic polynucleotide sequence and/or a composite polynucleotide sequences (e.g., a combination of the above).

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As used herein the phrase "complementary polynucleotide sequence" refers to a sequence, which results from reverse transcription of messenger RNA using a reverse transcriptase or any other RNA dependent DNA polymerase. Such a sequence

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can be subsequently amplified in vivo or in vitro using a DNA dependent DNA polymerase.

As used herein the phrase "genomic polynucleotide sequence" refers to a sequence derived (isolated) from a chromosome and thus it represents a contiguous portion of a chromosome.

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As used herein the phrase "composite polynucleotide sequence" refers to a sequence, which is at least partially complementary and at least partially genomic. A composite sequence can include some exonal sequences required to encode the polypeptide of the present invention, as well as some intronic sequences interposing therebetween. The intronic sequences can be of any source, including of other genes, and typically will include conserved splicing signal sequences. Such intronic sequences may further include cis acting expression regulatory elements.

According to a preferred embodiment of this aspect of the present invention, the nucleic acid sequence is as set forth in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 19, 21, 22, 23, 24, 25 or 26.

According to another preferred embodiment of this aspect of the present invention, the isolated polynucleotide is as set forth in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or 27.

According to yet another preferred embodiment of this aspect of the present invention, the polypeptide is as set forth in SEQ ID NO: 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 95 or 96.

According to still another preferred embodiment of this aspect of the present invention, the amino acid sequence is as set forth in SEQ ID NO: 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 95 or 96.

The isolated polynucleotides of this aspect of the present invention can also be qualified using a hybridization assay by incubating the isolated polynucleotides described above in the presence of oligonucleotide probe or primer under moderate to stringent hybridization conditions.

Moderate to stringent hybridization conditions are characterized by a hybridization solution such as containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and  $5 \times 10^6$  cpm  $^{32}$ P labeled probe, at 65 °C, with a final wash solution of  $0.2 \times SSC$  and 0.1 % SDS and final wash at 65 °C and whereas moderate hybridization is

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effected using a hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x  $10^6$  cpm  $^{32}$ P labeled probe, at 65 °C, with a final wash solution of 1 x SSC and 0.1 % SDS and final wash at 50 °C.

Thus, the present invention encompasses nucleic acid sequences described hereinabove; fragments thereof, sequences hybridizable therewith, sequences homologous thereto, sequences encoding similar polypeptides with different codon usage, altered sequences characterized by mutations, such as deletion, insertion or substitution of one or more nucleotides, either naturally occurring or man induced, either randomly or in a targeted fashion.

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Since the polynucleotide sequences of the present invention encode previously unidentified polypeptides, the present invention also encompasses novel polypeptides or portions thereof, which are encoded by the isolated polynucleotides and respective nucleic acid fragments thereof described hereinabove.

Thus, the present invention also encompasses polypeptides encoded by the polynucleotide sequences of the present invention. The amino acid sequences of these novel polypeptides are set forth in SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96.

The present invention also encompasses homologues of these polypeptides, such homologues can be at least about 70 %, at least about 75 %, at least about 80 %, at least about 81 %, at least about 82 %, at least about 83 %, at least about 84 %, at least about 85 %, at least about 86 %, at least about 87 %, at least about 88 %, at least about 89 %, at least about 90 %, at least about 91 %, at least about 92 %, at least about 93 %, at least about 94 %, at least about 95 %, at least about 96 %, at least about 97 %, at least about 98 %, at least about 99 %, or more say 100 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96.

The present invention also encompasses fragments of the above described polypeptides and polypeptides having mutations, such as deletions, insertions or substitutions of one or more amino acids, either naturally occurring or man induced, either randomly or in a targeted fashion.

The ability of polynucleotides of the present invention and their products to regulate cotton fiber development can be determined directly on at least one structural parameter of a cotton fiber such as fiber length or fiber finesse, or fiber growth rate

(further described hereinbelow). However cotton fiber development can also determined indirectly such as by plant model systems for cotton fiber development. For example, its is well established that trichome cells and root hairs share common characteristics with cotton fiber cells, and as such can be used as model systems for cotton fiber development [Reviewed in Wagner. G.J. et. al. (2004)], as demonstrated in details in Example 12 of the Examples section which follows.

By analyzing expression profiles, the present inventors were able to determine the involvement of the biomolecular sequences (i.e., polynucleotides and polypeptides) of the present invention in fiber initiation and/or elongation. These results were further substantiated by establishing a correlation between gene expression and fiber length (see Example 7).

These results suggest that biomolecular sequences of the present invention (e.g., polynucleotides, polypeptides, promoters, oligonucleotides, antibodies, also referred to herein as agents) can be used to improve fiber quality and/or yield of a fiber producing plant.

Thus, according to yet another aspect of the present invention there is provided a method of improving fiber quality and/or yield of a fiber producing plant.

The method of this aspect of the present invention is effected by regulating an expression level or activity of at least one polynucleotide or polypeptide of the present invention (described hereinabove) in the fiber producing plant, thereby improving the quality and/or yield of the fiber producing plant.

As used herein the phrase "fiber producing plant" refers to plants that share the common feature of having an elongated shape and abundant cellulose in thick cell walls, typically termed as secondary walls. Such walls may or may not be lignified, and the protoplast of such cells may or may be viable at maturity. Such fibers have many industrial uses, for example in lumber and manufactured wood products, paper, textiles, sacking and boxing material, cordage, brushes and brooms, filling and stuffing, caulking, reinforcement of other materials, and manufacture of cellulose derivatives.

According to a preferred embodiment of this aspect of the present invention the fiber producing plant is cotton.

The term "fiber" is usually inclusive of thick-walled conducting cells such as vessels and tracheids and to fibrillar aggregates of many individual fiber cells. Hence,

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the term "fiber" refers to (a) thick-walled conducting and non-conducting cells of the xylem; (b) fibers of extraxylary origin, including those from phloem, bark, ground tissue, and epidermis; and (c) fibers from stems, leaves, roots, seeds, and flowers or inflorescences (such as those of Sorghum vulgare used in the manufacture of brushes and brooms).

Example of fiber producing plants, include, but are not limited to, agricultural crops such as cotton, silk cotton tree (Kapok, Ceiba pentandra), desert willow, creosote bush, winterfat, balsa, kenaf, roselle, jute, sisal abaca, flax, corn, sugar cane, hemp, ramie, kapok, coir, bamboo, spanish moss and Agave spp. (e.g. sisal).

As used herein the phrase "fiber quality" refers to at least one fiber parameter which is agriculturally desired, or required in the fiber industry (further described hereinbelow). Examples of such parameters, include but are not limited to, fiber length, fiber strength, fiber fitness, fiber weight per unit length, maturity ratio and uniformity (further described hereinbelow.

Cotton fiber (lint) quality is typically measured according to fiber length, strength and fineness. Accordingly, the lint quality is considered higher when the fiber is longer, stronger and finer.

As used herein the phrase "fiber yield" refers to the amount or quantity of fibers produced from the fiber producing plant.

As used herein the term "improving" refers to at least about 5 %, at least about 10 %, at least about 15 %, at least about 20 %, at least about 30 %, at least about 40 %, at least about 50 %, change in fiber quality/yield as compared to a native plant (i.e., not modified with the biomolecular sequences of the present invention).

As used herein the term "regulating" refers to up regulating, down regulating or a combination thereof. For example, when an increase in fiber number is desired the present invention can be effected by upregulating at least one polynucleotide of the present invention, which is involved in fiber initiation (e.g., SEQ ID NOs: 4, 10, 9, 12, 16 and 25). Alternatively, when short fibers are desired such as for example, in corn, then the present invention is effected by down regulating at least one polynucleotide of the present invention which is involved in fiber elongation (e.g., SEQ ID NOs. 1, 2, 3, 5, 6, 7, 17, 18, 19, 20, 21, 22, 23, 24 and 27). Alternatively, the present invention can be effected by upregulating expression of at least one polynucleotide (such as involved in fiber elongation) and down regulating at least one

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polynucleotide (such as involved in fiber initiation) of the polynucleotides of the present invention. In this manner it is feasible to obtain a fiber producing plant with improved fiber yield of each of short length.

Up regulating an expression level of at least one of the polynucleotides of the present invention can be effected at the genomic level (e.g., activation of transcription by means of promoters, enhancers, or other regulatory elements), at the transcript level, or at the protein level.

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Following is a non-comprehensive list of agents capable of upregulating the expression level and/or activity of the biomolecular sequences (i.e., nucleic acid or protein sequences) of the present invention.

An agent capable of upregulating expression of a polynucleotide of interest may be an exogenous polynucleotide sequence designed and constructed to express at least a functional portion thereof (e.g., improving fiber yield/quality, increasing biomass etc.). Accordingly, the exogenous polynucleotide sequence may be a DNA or RNA sequence encoding a polypeptide molecule, capable of improving fiber yield or quantity. Alternatively, the exogenous polynucleotide may be a cis-acting regulatory region (e.g., SEQ ID NO: 74, 75, 85, 88 or 91) which may be introduced into the plant to increase expression of any polynucleotide which is involved in fiber development (e.g., sucrose phosphate synthase, as described in U.S. Pat. No. 6,472,588).

To express exogenous polynucleotides in plant cells, a polynucleotide sequence of the present invention is preferably ligated into a nucleic acid construct suitable for plant cell expression. Such a nucleic acid construct includes a cis-acting regulatory region such as a promoter sequence for directing transcription of the polynucleotide sequence in the cell in a constitutive or inducible manner. The promoter may be homologous or heterologous to the transformed plant/cell.

Preferred promoter sequences which can be used in accordance with this aspect of the present invention are endothelial cell promoters.

For example, promoter sequences of each of the polynucleotide sequences of the present invention may be preferably used in the nucleic acid constructs of the present invention.

According to a preferred embodiment of this aspect of the present invention the promoter is at least about 80 %, at least about 81 %, at least about 82 %, at least about 83 %, at least about 84 %, at least about 85 %, at least about 86 %, at least

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about 87 %, at least about 88 %, at least about 89 %, at least about 90 %, at least about 91 %, at least about 92 %, at least about 93 %, at least about 94 %, at least about 95 %, at least about 96 %, at least about 97 %, at least about 98 %, at least about 99 %, or 100 % identical to SEQ ID NO. 85 or 91, which is capable of regulating expression of at least one polynucleotide sequence operably linked thereto in an ovule endothelial cell (i.e., capable of exerting a regulatory effect on the coding sequence linked thereto).

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As is clearly illustrated in the Examples section which follows, such promoter sequences are capable of regulating expression of a coding nucleic acid sequence (e.g., GUS) operably linked thereto.

Other examples of cotton fiber-enhanced promoters include those of the cotton fiber-expressed genes E6 (John et al., Plant Mol. Biol., 30:297-306 (1996) and John et al., Proc. Natl. Acad. Sci., 93:12768-12773 (1996) e), H6 (John et al., Plant Physiol., 108:669-676, (1995)), FbL2A (Rinehart et al., Plant Physiol., 112:1331-1341 (1996) and John et al, Proc. Natl. Acad. Sci. USA, 93:12768-12773 (1996)), rac (Delmer et al., Mol. Gen. Genet., 248:43-51 (1995)); CelA (Pear et al., Proc. Natl. Acad. Sci USA, 93:12637-12642 (1996)); CAP (Kawai et al., Plant Cell Physiol. 39:1380-1383 (1998)); ACP (Song et al., Biochim. Biophys. Acta 1351:305-312 (1997); and LTP (Ma et al., Biochim. Biophys. Acta 1344:111-114 (1997)). Other cotton fiber specific promoters are disclosed in U.S. Pat. No. 5,495,070.

Other promoters which can be used in accordance with this aspect of the present invention are those that ensure expression only in specified organs, such as the leaf, root, tuber, seed, stem, flower or specified cell types such as parenchyma, epidermal, trichome or vascular cells.

Preferred promoters for enhancing expression in trichome cells are disclosed in WO 2004/111183, to Evogene Ltd.

Preferred promoters enhancing expression in vascular tissue include the CAD 2 promoter (Samaj et al., Planta, 204:437-443 (1998)), the Pt4C11 promoter (Hu et al., Proc. Natl. Acad. Sci. USA, 95:5407-5412 (1998)), the C4H promoter (Meyer et al., Proc. Natl. Acad. Sci. USA, 95:6619-6623 (1998)), the PtX3H6 and PtX14A9 promoters (Loopstra et al., Plant Mol. Biol., 27:277-291 (1995)), the RolC promoter (Graham, Plant Mol. Biol., 33:729-735 (1997)), the Hvhsp17 promoter (Raho et al., J.

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Expt. Bot., 47:1587-1594 (1996)), and the COMT promoter (Capellades et al., Plant Mol. Biol., 31:307-322 (1996)).

Preferred promoters enhancing expression in stem tissue include pith promoters (Datta, Theor. Appl. Genet., 97:20-30 (1998) and Ohta et al., Mol. Gen. Genet., 225:369-378 (1991)), and the anionic peroxidase promoter (Klotz et al., Plant Mol. Biol., 36:509-520 (1998)). Preferred promoters enhancing expression in phloem, cortex and cork, but not xylem or pith, include the Psam-1 promoter (Mijnsbrugge et al., Plant and Cell Physiol., 37:1108-1115 (1996)).

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Preferred promoters enhancing expression in seeds include the phas promoter (Geest et al., Plant Mol. Biol. 32:579-588 (1996)); the GluB-1 promoter (Takaiwa et al., Plant Mol. Biol. 30:1207-1221 (1996)); the gamma-zein promoter (Torrent et al. Plant Mol. Biol. 34:139-149 (1997)), and the oleosin promoter (Sarmiento et al., The Plant Journal 11:783-796 (1997)).

Other promoter sequences which mediate constitutive, inducible, tissue-specific or developmental stage-specific expression are disclosed in WO 2004/081173 to Evogene Ltd.

Truncated or synthetic promoters including specific nucleotide regions conferring tissue-enhanced expression may also be used, as exemplified by identification of regulatory elements within larger promoters conferring xylemenhanced expression (Seguin et al., Plant Mol. Biol., 35:281-291 (1997); Torres-Schumann et al., The Plant Journal, 9:283-296 (1996); and Leyva et al., The Plant Cell, 4:263-271 (1992)).

The nucleic acid construct can be, for example, a plasmid, a bacmid, a phagemid, a cosmid, a phage, a virus or an artificial chromosome. Preferably, the nucleic acid construct of the present invention is a plasmid vector, more preferably a binary vector.

The phrase "binary vector" refers to an expression vector which carries a modified T-region from Ti plasmid, enable to be multiplied both in *E. coli* and in *Agrobacterium* cells, and usually comprising reporter gene(s) for plant transformation between the two boarder regions. A binary vector suitable for the present invention includes pBI2113, pBI121, pGA482, pGAH, pBIG, pBI101 (Clonetech), pPI (see Example 5 of the Examples section which follows) or modifications thereof.

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The nucleic acid construct of the present invention can be utilized to transform a host cell (e.g., bacterial, plant) or plant.

As used herein, the terms "transgenic" or "transformed" are used interchangeably referring to a cell or a plant into which cloned genetic material has been transferred.

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In stable transformation, the nucleic acid molecule of the present invention is integrated into the plant genome, and as such it represents a stable and inherited trait. In transient transformation, the nucleic acid molecule is expressed by the cell transformed but not integrated into the genome, and as such represents a transient trait.

There are various methods of introducing foreign genes into both monocotyledonous and dicotyledonous plants (Potrykus, I. (1991). Annu Rev Plant Physiol Plant Mol Biol 42, 205-225; Shimamoto, K. et al. (1989). Fertile transgenic rice plants regenerated from transformed protoplasts. Nature (1989) 338, 274-276).

The principal methods of the stable integration of exogenous DNA into plant genomic DNA includes two main approaches:

- (i) Agrobacterium-mediated gene transfer. See: Klee, H. J. et al. (1987). Annu Rev Plant Physiol 38, 467-486; Klee, H. J. and Rogers, S. G. (1989). Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes, pp. 2-25, J. Schell and L. K. Vasil, eds., Academic Publishers, San Diego, Cal.; and Gatenby, A. A. (1989). Regulation and Expression of Plant Genes in Microorganisms, pp. 93-112, Plant Biotechnology, S. Kung and C. J. Arntzen, eds., Butterworth Publishers, Boston, Mass.
- (ii) Direct DNA uptake. See, e.g.: Paszkowski, J. et al. (1989). Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes, pp. 52-68, J. Schell and L. K. Vasil, eds., Academic Publishers, San Diego, Cal.; and Toriyama, K. et al. (1988). Bio/Technol 6, 1072-1074 (methods for direct uptake of DNA into protoplasts). See also: Zhang et al. (1988). Plant Cell Rep 7, 379-384; and Fromm, M. E. et al. (1986). Stable transformation of maize after gene transfer by electroporation. Nature 319, 791-793 (DNA uptake induced by brief electric shock of plant cells). See also: Klein et al. (1988). Bio/Technology 6, 559-563; McCabe, D. E. et al. (1988). Stable transformation of soybean (Glycine max) by particle acceleration. Bio/Technology 6, 923-926; and Sanford, J. C. (1990). Biolistic

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plant transformation. Physiol Plant 79, 206-209 (DNA injection into plant cells or tissues by particle bombardment). See also: Neuhaus, J. M. et al. (1987). Theor Appl Genet 75, 30-36; and Neuhaus, J. M. and Spangenberg, G. C. (1990). Physiol Plant 79, 213-217 (use of micropipette systems). See U.S. Pat. No. 5,464,765 (glass fibers or silicon carbide whisker transformation of cell cultures, embryos or callus tissue). See also: DeWet, J. M. J. et al. (1985). "Exogenous gene transfer in maize (Zea mays) using DNA-treated pollen," Experimental Manipulation of Ovule Tissue, G. P. Chapman et al., eds., Longman, New York-London, pp. 197-209; and Ohta, Y. (1986). High-Efficiency Genetic Transformation of Maize by a Mixture of Pollen and Exogenous DNA. Proc Natl Acad Sci USA 83, 715-719 (direct incubation of DNA with germinating pollen).

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The Agrobacterium-mediated system includes the use of plasmid vectors that contain defined DNA segments which integrate into the plant genomic DNA. Methods of inoculation of the plant tissue vary depending upon the plant species and the Agrobacterium delivery system. A widely used approach is the leaf-disc procedure, which can be performed with any tissue explant that provides a good source for initiation of whole-plant differentiation (Horsch, R. B. et al. (1988). "Leaf disc transformation." Plant Molecular Biology Manual A5, 1-9, Kluwer Academic Publishers, Dordrecht). A supplementary approach employs the Agrobacterium delivery system in combination with vacuum infiltration. The Agrobacterium system is especially useful for in the creation of transgenic dicotyledenous plants.

There are various methods of direct DNA transfer into plant cells. In electroporation, the protoplasts are briefly exposed to a strong electric field, opening up mini-pores to allow DNA to enter. In microinjection, the DNA is mechanically injected directly into the cells using micropipettes. In microparticle bombardment, the DNA is adsorbed on microprojectiles such as magnesium sulfate crystals or tungsten particles, and the microprojectiles are physically accelerated into cells or plant tissues.

Following stable transformation, plant propagation occurs. The most common method of plant propagation is by seed. The disadvantage of regeneration by seed propagation, however, is the lack of uniformity in the crop due to heterozygosity, since seeds are produced by plants according to the genetic variances governed by Mendelian rules. In other words, each seed is genetically different and each will grow with its own specific traits. Therefore, it is preferred that the regeneration be effected

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such that the regenerated plant has identical traits and characteristics to those of the parent transgenic plant. The preferred method of regenerating a transformed plant is by micropropagation, which provides a rapid, consistent reproduction of the transformed plants.

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Micropropagation is a process of growing second-generation plants from a single tissue sample excised from a selected parent plant or cultivar. This process permits the mass reproduction of plants having the preferred tissue and expressing a fusion protein. The newly generated plants are genetically identical to, and have all of the characteristics of, the original plant. Micropropagation allows for mass production of quality plant material in a short period of time and offers a rapid multiplication of selected cultivars with preservation of the characteristics of the original transgenic or transformed plant. The advantages of this method of plant cloning include the speed of plant multiplication and the quality and uniformity of the plants produced.

Micropropagation is a multi-stage procedure that requires alteration of culture medium or growth conditions between stages. The micropropagation process involves four basic stages: stage one, initial tissue culturing; stage two, tissue culture multiplication; stage three, differentiation and plant formation; and stage four, greenhouse culturing and hardening. During stage one, the tissue culture is established and certified contaminant-free. During stage two, the initial tissue culture is multiplied until a sufficient number of tissue samples are produced to meet production goals. During stage three, the newly grown tissue samples are divided and grown into individual plantlets. At stage four, the transformed plantlets are transferred to a greenhouse for hardening where the plants' tolerance to light is gradually increased so that they can continue to grow in the natural environment.

Although stable transformation is presently preferred, transient transformation of, for instance, leaf cells, meristematic cells, or the whole plant is also envisaged by the present invention.

Transient transformation can be effected by any of the direct DNA transfer methods described above or by viral infection using modified plant viruses.

Viruses that have been shown to be useful for the transformation of plant hosts include cauliflower mosaic virus (CaMV), tobacco mosaic virus (TMV), and baculovirus (BV). Transformation of plants using plant viruses is described in, for example: U.S. Pat. No. 4,855,237 (bean golden mosaic virus, BGMV); EPA 67,553

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(TMV); Japanese Published Application No. 63-14693 (TMV); EPA 194,809 (BV); EPA 278,667 (BV); and Gluzman, Y. et al. (1988). Communications in Molecular Biology: Viral Vectors, Cold Spring Harbor Laboratory, New York, pp. 172-189. The use of pseudovirus particles in expressing foreign DNA in many hosts, including plants, is described in WO 87/06261.

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Construction of plant RNA viruses for the introduction and expression of non-viral exogenous nucleic acid sequences in plants is demonstrated by the above references as well as by: Dawson, W. O. et al. (1989). A tobacco mosaic virus-hybrid expresses and loses an added gene. Virology 172, 285-292; French, R. et al. (1986) Science 231, 1294-1297; and Takamatsu, N. et al. (1990). Production of enkephalin in tobacco protoplasts using tobacco mosaic virus RNA vector. FEBS Lett 269, 73-76.

If the transforming virus is a DNA virus, one skilled in the art may make suitable modifications to the virus itself. Alternatively, the virus can first be cloned into a bacterial plasmid for ease of constructing the desired viral vector with the foreign DNA. The virus can then be excised from the plasmid. If the virus is a DNA virus, a bacterial origin of replication can be attached to the viral DNA, which is then replicated by the bacteria. Transcription and translation of the DNA will produce the coat protein, which will encapsidate the viral DNA. If the virus is an RNA virus, the virus is generally cloned as a cDNA and inserted into a plasmid. The plasmid is then used to make all of the plant genetic constructs. The RNA virus is then transcribed from the viral sequence of the plasmid, followed by translation of the viral genes to produce the coat proteins which encapsidate the viral RNA.

Construction of plant RNA viruses for the introduction and expression in plants of non-viral exogenous nucleic acid sequences, such as those included in the construct of the present invention, is demonstrated in the above references as well as in U.S. Pat. No. 5,316,931.

In one embodiment, there is provided for insertion a plant viral nucleic acid, comprising a deletion of the native coat protein coding sequence from the viral nucleic acid, a non-native (foreign) plant viral coat protein coding sequence, and a non-native promoter, preferably the subgenomic promoter of the non-native coat protein coding sequence, and capable of expression in the plant host, packaging of the recombinant plant viral nucleic acid, and ensuring a systemic infection of the host by the recombinant plant viral nucleic acid. Alternatively, the native coat protein coding

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sequence may be made non-transcribable by insertion of the non-native nucleic acid sequence within it, such that a non-native protein is produced. The recombinant plant viral nucleic acid construct may contain one or more additional non-native subgenomic promoters. Each non-native subgenomic promoter is capable of transcribing or expressing adjacent genes or nucleic acid sequences in the plant host and incapable of recombination with each other and with native subgenomic promoters. In addition, the recombinant plant viral nucleic acid construct may contain one or more *cis*-acting regulatory elements, such as enhancers, which bind a transacting regulator and regulate the transcription of a coding sequence located downstream thereto. Non-native nucleic acid sequences may be inserted adjacent to the native plant viral subgenomic promoter or the native and non-native plant viral subgenomic promoters if more than one nucleic acid sequence is included. The non-native nucleic acid sequences are transcribed or expressed in the host plant under control of the subgenomic promoter(s) to produce the desired products.

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In a second embodiment, a recombinant plant viral nucleic acid construct is provided as in the first embodiment except that the native coat protein coding sequence is placed adjacent to one of the non-native coat protein subgenomic promoters instead of adjacent to a non-native coat protein coding sequence.

In a third embodiment, a recombinant plant viral nucleic acid construct is provided comprising a native coat protein gene placed adjacent to its subgenomic promoter and one or more non-native subgenomic promoters inserted into the viral nucleic acid construct. The inserted non-native subgenomic promoters are capable of transcribing or expressing adjacent genes in a plant host and are incapable of recombination with each other and with native subgenomic promoters. Non-native nucleic acid sequences may be inserted adjacent to the non-native subgenomic plant viral promoters such that said sequences are transcribed or expressed in the host plant under control of the subgenomic promoters to produce the desired product.

In a fourth embodiment, a recombinant plant viral nucleic acid construct is provided as in the third embodiment except that the native coat protein coding sequence is replaced by a non-native coat protein coding sequence.

Viral vectors are encapsidated by expressed coat proteins encoded by recombinant plant viral nucleic acid constructs as described hereinabove, to produce a recombinant plant virus. The recombinant plant viral nucleic acid construct or

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recombinant plant virus is used to infect appropriate host plants. The recombinant plant viral nucleic acid construct is capable of replication in a host, systemic spread within the host, and transcription or expression of one or more foreign genes (isolated nucleic acid) in the host to produce the desired protein.

In addition to the above, the nucleic acid molecule of the present invention can also be introduced into a chloroplast genome thereby enabling chloroplast expression.

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A technique for introducing exogenous nucleic acid sequences to the genome of the chloroplasts is known. This technique involves the following procedures. First, plant cells are chemically treated so as to reduce the number of chloroplasts per cell to about one. Then, the exogenous nucleic acid is introduced into the cells preferably via particle bombardment, with the aim of introducing at least one exogenous nucleic acid molecule into the chloroplasts. The exogenous nucleic acid is selected by one ordinarily skilled in the art to be capable of integration into the chloroplast's genome via homologous recombination, which is readily effected by enzymes inherent to the chloroplast. To this end, the exogenous nucleic acid comprises, in addition to a gene of interest, at least one nucleic acid sequence derived from the chloroplast's genome. In addition, the exogenous nucleic acid comprises a selectable marker, which by sequential selection procedures serves to allow an artisan to ascertain that all or substantially all copies of the chloroplast genome following such selection include the exogenous nucleic acid. Further details relating to this technique are found in U.S. Pat. Nos. 4,945,050 and 5,693,507, which are incorporated herein by reference. A polypeptide can thus be produced by the protein expression system of the chloroplast and become integrated into the chloroplast's inner membrane.

Downregulation of a gene of interest can be effected on the genomic and/or the transcript level using a variety of molecules that interfere with transcription and/or translation (e.g., antisense, siRNA), or on the protein level using, e.g., antibodies, immunization techniques and the like.

For example, an agent capable of downregulating an activity of a polypeptide of interest is an antibody or antibody fragment capable of specifically binding a polypeptide of the present invention. Preferably, the antibody specifically binds at least one epitope of the polypeptide of interest. As used herein, the term "epitope" refers to any antigenic determinant on an antigen to which the paratope of an antibody binds.

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Down –regulation at the RNA level can be effected by RNA-based silencing strategies which are effective in plants. See for example, Kusaba (2004) RNA interference in crop plants. Curr. Opin. Biotechnol. 15(2):139-43; Matzke (2001) RNA based silencing strategies in plants. Curr. Opin. Genet. 11:221-7.

For example, an agent capable of downregulating a polynucleotide of interest is a small interfering RNA (siRNA) molecule in the process of RNA interference (RNAi).

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dsRNAs can be delivered to plants in several ways (reviewed in Waterhouse P. Helliwell C. 2003. Exploring plant genomes by RNA-induced gene silencing. Nature Genet 4: 29-38): microprojectile bombardment with dsRNA or introncontaining hairpin RNA (ihpRNA)-expressing vectors; infiltration of plant tissue with an Agrobacterium strain carrying a T-DNA expressing an ihpRNA transgene; virus induced gene silencing (VIGS), in which the target sequence is integrated into viral sequences which are used to infect the plant, or are expressed from Agrobacteriumintroduced transgenes, and by stable transformation with ihpRNA expressing transgenes. The various RNAi techniques each have advantages and disadvantages with respect to how persistent their effect is and the range of plants to which they can be applied, e.g. bombardment can be applied to any plant, but produces only transient effects. Alternatively, transformation with ihpRNA-expressing transgenes provides stable and heritable gene silencing, but requires efficient plant transformation techniques. ihpRNA transgenes have been shown to be very effective for a wide range of target genes in various plant species (reviewed in Waterhouse P, Helliwell C. 2003. Exploring plant genomes by RNA-induced gene silencing. Nature Genet 4: 29-38; Wesley S, Helliwell C, Smith N, et al. 2001. Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J 27: 581-590), indicating that the RNAi mechanism is probably conserved in all plant species. This is supported by a recent report of RNAi in the non-vascular moss Physcomitrella patens (Bezanilla M, Pan A, Quatrano R. 2003. RNA interference in the moss Physcomitrella patens. Plant Physiol 133: 470-474).

Antisense genetic constructs for fiber specific promoters (e.g., for SEQ ID NO: 85, 91) can be used to inhibit or lessen the expression of one or more fiber genes in fiber cells. The use of antisense constructs is described in U.S. Pat. No. 5,495,070 and in Smith, et al. Nature 334 724-726, 1988; Bird, et al. Bio/Technology 9: 635-

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639, 1991; Van der Krol, et al. Gene 72: 45-50, 1988.

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It will be appreciated that the generation of fiber producing plant of desired traits according to the present invention can also be effected by crossing each of the above genetically modified plants with wild type, hybrid or transgenic plants, using methods which are well known in the art.

Once the transgenic planta of the present invention are generated, fibers are harvested (for example by mechanical picking and/or hand-stripping) and fiber yield and quality is determined.

The following describes methods of qualifying cotton fibers.

Fiber length - Instruments such as a fibrograph and HVI (high volume instrumentation) systems are used to measure the length of the fiber. HVI instruments compute length in terms of "mean" and "upper half mean" (UHM) length. The mean is the average length of all the fibers while UHM is the average length of the longer half of the fiber distribution.

Fiber strength – As mentioned, fiber strength is usually defined as the force required to break a bundle of fibers or a single fiber. In HVI testing the breaking force is converted to "grams force per tex unit." This is the force required to break a bundle of fibers that is one tex unit in size. In HVI testing the strength is given in grams per tex units (grams/tex). Fibers can be classified as low strength (e.g., 19-22 gms/tex), average strength (e.g., 23-25 gms/tex), high strength (e.g., 26-28 gms/tex), and very high strength (e.g., 29-36 gms/tex).

Micronaire - The micronaire reading of a fiber is obtained from a porous air flow test. The test is conducted as follows. A weighed sample of cotton is compressed to a given volume and controlled air flow is passed through the sample. The resistance to the air flow is read as micronaire units. The micronaire readings reflects a combination of maturity and fineness. Since the fiber diameter of fibers within a given variety of cotton is fairly consistent, the micronaire index will more likely indicate maturity variation rather than variations in fineness. A micronaire reading of 2.6-2.9 is low while 3.0-3.4 is below average, 3.5-4.9 is average and 5.0 and up are high. For most textile applications a micronaire of 3.5-4.9 is used. Anything higher than this is usually not desirable. It will be appreciated though, that different applications require different fiber properties. Thus, it is understood that a fiber property that is disadvantageous in one application might be advantageous in another.

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As is illustrated in the Examples section, which follows, biomolecular sequences of the present invention are capable of increasing trichome/leaf hair number and length, as well as seed hair. As such biomolecular sequences of the present invention can be used to generate transgenic plants with increased trichome number/length which better deter herbivores, guide the path of pollinators, or affect photosynthesis, leaf temperature, or water loss through increased light reflectance. Additionally such transgenic plants may be used for the compartmentalized production of recombinant proteins and chemicals in trichomes, as described in details in WO 2004/111183 to Evogene Ltd.

Interestingly and unexpectedly, the present inventors found that polynucleotide sequences of the present invention are capable of increasing a biomass of a plant. It will be appreciated that the ability of the polypeptides of the present invention to increase plant yield/biomass/vigor is inherent to their ability to promote the increase in plant cell-size or volume (as described herein).

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Thus, the present invention also envisages a method of increasing a biomass/vigor/yield of a plant (coniferous plants, moss, algae, monocot or dicot, as well as other plants listed in www.nationmaster.com/encyclopedia/Plantae). This is effected by regulating expression and/or activity of at least one of the polynucleotides of the present invention, as described above.

As used herein the phrase "plant biomass" refers to the amount or quantity of tissue produced from the plant in a growing season, which could also determine or affect the plant yield or the yield per growing area.

As used herein the phrase "plant vigor" refers to the amount or quantity of tissue produced from the plant in a given time. Hence increase vigor could determine or affect the plant yield or the yield per growing time or growing area.

As used herein the phrase "plant yield" refers to the amount or quantity of tissue produced and harvested as the plant produced product. Hence increase yield could affect the economic benefit one can obtain from the plant in a certain growing are and/or growing time.

Thus, the present invention is of high agricultural value for promoting the yield of commercially desired crops (e.g., biomass of vegetative organ such as poplar wood, or reproductive organ such as number of seeds or seed biomass).

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As used herein the term "about" refers to  $\pm$  10 %.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

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#### **EXAMPLES**

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M.

J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

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#### EXAMPLE 1

# In silico identification of cotton genes involved in fiber formation Experimental Procedures

Interspecies comparison of expressed sequences- Two main tools were used during the data mining stage. Large numbers of gene profiles were queried from an ORACLE database housing Compugen's GeneCarta platform (Compugen Ltd. Israel). This data was loaded into MicroSoft Excel spreadsheets for further manual refinement. Using this data a cross species genomic comparison was effected, aiming at defining organs from other plant species for which publically available EST libraries can be used both as models and as new sources of information to define new genes with key role in fiber formation (Figure 1). This comparison analysis used mainly the cotton, arabidopsis and tomato databases.

Clustering and inter-species clustering of EST sequences - The cotton genomic database included less than 50,000 ESTs (Genbank release #135) originating primarily from two species Gossypium arboreum ( $\sim$  35,000 ESTs) and Gossypium hirsutum L. ( $\sim$  9,000 ESTs, Table 1, below). These ESTs were clustered and assembled using the LEADS<sup>TM</sup> software platform (Compugen Ltd, Israel) in two alternative approaches.

In the first approach, the ESTs from two species were clustered and assembled together (thereby mimicking the evolutionary process since G. arboreum is an

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ancestor of *G. hirsutum*). This process revealed 6478 clusters among them 3243 new clusters (without mRNA in the public database) that were defined as high quality clusters (Table 1, below).

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In the second approach, ESTs from each species were clustered and assembled separately. Comparison between the two approaches showed that using the first approach adds valuable information to the cotton clusters without a significant bias in the analysis. The tomato genomic database contains 126,156 ESTs originating from about 30 well defined libraries that through the clustering and assembling process revealed 14034 clusters of which a large group of 12787 new high quality clusters of arabidopsis includes 99417 data genomic 1). The full length cDNA (Rikken and genbank (ftp://ftp.ncbi.nih.gov/genbank/), 8573 mRNAs ftp://ftp.ncbi.nih.gov/genbank/) and the entire DNA sequence. Using the LEADS software 23,148 clusters and 6777 singeltones (Single ESTs which no other EST was clustered therewith) were revealed, all of which were supported by ESTs sequences, contrary to the public consortium (TAIR, www.arabidopsis.org/).

EST libraries from other plants and organs that share similar biological processes as cotton fiber were sought. Such ESTs are expected to serve as models and as new information sources for the identification of genes which are involved in the fiber development. To this end, a list of known genes that are suspected to be involved in fiber formation was generated. These genes originated from arabidopsis and were shown in various studies to have a key role in trichome formation (i.e., GL2, CPC, bHLH, TTG1, GL1, reviewed in Larkin J.C. et.al. 2003, Schellmann S. et al. 2002). Extensive comparative genomic analysis revealed that tomato genes, with high homology to cotton fiber genes and to arabidopsis trichome genes have a significant EST content in either leaf trichome and specific flower development libraries. Further analysis compared the genomic data of these three species — cotton, Arabidopsis and tomato (focusing on the tomato libraries mentioned above) as key parameters in the present database search (Figure 1).

Table 1
Genomic databases of Cotton, Tomato and Arabidopsis

Genomic databases of Cotton, Lomato and Arabidopsis						
Species	EST Lib description	EST count	mRNA	After LEADS (clusters)		
G. arboreum	Fiber 6DPA	37,276	12	16,294 clusters		
G. hirsutum	Fiber 7-10 DPA	7,944	236	on mixed		

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G. hirsutum	Flower ovule 1DPA	1,272	870	production*
L. esculentum	All libraries	115,859	7	25,678 clusters on mixed production
L. hirsutum	Trichome libraries	2,409	7	
L. pennellii	Trichome libraries	2,723	24,450	
A. thaliana	All libraries	160,698	mRNA	25,678 clusters

<sup>\*</sup>clusters derived from different species, cotton G. arboreum and G. hirsutum, tomato L. esculentum, L. hirsutum and L. pennellii

In silico identification of cotton genes with a role in fiber development To find whether tomato genomic data can be used as a relevant source of genomic data to study cotton fiber development an extensive genomic comparison was effected to identify both tomato and cotton genes that have high homology to key genes determining arabidopsis trichome development (e.g., GL2, CPC, bHLH, TTG1, GL1).

Homologous genes were identified in cotton and tomato. Because almost all cotton ESTs were produced from cotton fibers, it was impossible to do in-silico prediction of the expression profile of those genes. However, wide tissue sources used for the production of the tomato EST database enabled identification of tissues in which trichome specific genes are expressed.

In tomato it was revealed that both trichome and ovule ESTs are enriched in clusters representing trichome specific genes. Interestingly, it was found that cotton fibers are produced from ovule coat cells. As tomato seeds are covered with hairy like tissue, similarly to cotton seeds, it was postulated that those hairs are developmentally linked to trichome and cotton fiber formation.

In tomato ~1100 clusters were found to include at least one EST from trichome libraries. Among them about 1000 sequences included sequences also originating from tomato flower libraries (in which the ovule tissue is present). Comparing this group of genes to cotton data revealed ~2300 cotton genes with high homology to the tomato trichome genes. Mining the database using these two groups of genes together with other bioinformatic information [cross species homology, Gene Onthology (GO)] revealed 80 cotton clusters predicted to have a key role in fiber formation. Those genes were selected based on the following criteria:

Cotton clusters with at least 2 ESTs;

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Homology to tomato cluster with e-score higher than 1e-5;

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Homology to tomato cluster with at least one EST coming from trichome libraries or one EST coming from ovule containing tissues;

The following criteria were considered as advantageous although not necessary:

Large number of ESTs in a cluster;

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Transcription factor/ signal transduction proteins;

Gene annotation related to cell expansion, turgor pressure, cell-wall synthesis.

The new genes together with the control cotton genes known to be involved in fiber formation were further analysed for their RNA expression profile in cotton plants.

#### EXAMPLE 2

# mRNA expression analysis of genes identified according to the teachings of the present invention

To study the RNA expression profile of candidate genes identified as described in Example 1 above, a reverse transcription was effected followed by real time PCR (RT-qPCR).

#### **Experimental Procedures**

Quantitative Real time PCR analysis (qRT PCR) - To verify the levels of expression specificity and trait-association, Reverse Transcription following quantitative (Real-Time) PCR (RTqPCR) was effected. Total RNA was extracted at different stages of fiber development (from the day of anthesis till day 20 - post anthesis). To study the specificity of expression, RNA from other tissues of the cotton plants were collected and analysed for control expression (i.e., young leaves, young stems, mature stems, young roots, sepals, petals, and stamen). For this purpose, RNA was extracted from Cotton tissue using Hot Borate RNA Extraction protocol according to <a href="https://www.eeob.iastate.edu/faculty/WendelJ/ultramicrorna.html">www.eeob.iastate.edu/faculty/WendelJ/ultramicrorna.html</a> Reverse transcription was effected using 1.5 μg total RNA, using 300 U Super Script II Reverse Transcriptase enzyme (Invitrogen), 225ng random deoxynucleotide hexamers (Invitrogen), 500 μM dNTPs mix (Takara, Japan), 0.2 volume of x 5 RT buffer (Invitrogen), 0.01M DTT, 60U RNAsin (Promega), DEPC treated double distilled water was added up to 37.5 μl. RT reactions were incubated for 50 min at 42 °C,

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followed by 70 °C for 15 min. cDNA was diluted 1:20 in Tris EDTA, pH=8. 5mL of the diluted cDNA was used for qRT-PCR.

Quantitative RT-PCR was performed on cDNA (5 µL), using x1 SYBR GREEN PCR master mix (Applied Biosystems), forward and reverse primers 0.3 µM each. The ABI7000real-time PCR machine was used with the following conditions 50 °C for 2 min, 95 °C for 10 min, 40 times of 95 °C for 15 sec and 1 min at 60 °C, followed by 95 °C for 15 sec, 60 °C for 60 sec, and 70 times of 60 °C for 10 sec +0.5 °C increase in each cycle. For each gene, a standard curve was prepared from a pool of RTs from all samples, in 5 dilutions (dilutions - 1:60, 1:200, 1:600, 1:2000, 1:10000). The standard curve plot [ct (cycle threshold) vs. log (concentration)] should have R>=0.98 with an efficiency in the range of 100% +/- 5%. The levels of expression (Qty) measured in the qPCR were calculated using the efficiency (E) of the amplification reaction and the corresponding C.T. (the cycle at which the samples crossed the threshold) Qty=E-C.T.. The dissociation curves obtained were inspected for the absence of unwanted additional PCR products or primer-dimers. Reactions were repeated at least twice. The calculation method is based in the fact that the efficiencies of the reactions of the GOI (gene of interest) and of the housekeeping genes are similar.

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To normalize the expression level between the different tissues, specific primers were designed for specifically hybridizing with the following housekeeping genes: Actin (GenBank Accession No. D88414 SEQ ID NO: 28, Forward and reverse primers are set forth in SEQ ID NO: 68 and 69, respectively), GAPDH (GenBank Accession No. COTCWPPR, partial sequence, SEQ ID NO: 29, Forward and reverse primers are set forth in SEQ ID NO: 97 and 98, respectively), and RPL19 (GenBank Accession No. AI729179, SEQ ID NO: 30, Forward and reverse primers are set forth in SEQ ID NO: 99 and 100, respectively).

Using this methodology it was possible to identify genes that show elevated expression during fiber elongation, as well as genes that show unique cotton fiber specificity. Genes that showed elevated expression during anthesis that decreases during fiber elongation were considered good candidates to be involved in fiber differentiation and initiation. Notably, the above-described quantification methodology did not provide absolute expression levels, but provided good parameters for scoring the relative gene expression along fiber development as

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differences as high as over 1000 fold in the maximal levels of expression reached by different genes were detected (Table 2, below).

## Results

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88 cotton genes were evaluated for expression profile in different tissues of cotton (*Gossypium hirsutum*, var Acala). According to the gene expression results, 23 genes were predicted to improve fiber yield and quality. Expression profile of all the candidate genes are presented in Table 2.

Table 2

									38	3														
young	0.202	0.025	0.063	0.003	0.044	0.049	0.012	0.000	0.067	0.762	0.000	0.007	0.004	0.047	1.294	0.480	0.464	2.759	0.000	0.003	1.089	0.000	0.035	0.007
young roots	0.002	0.068	0.037	0.003	0.037	0.000	0.000	0.000	0.069	1.308	•	0.001	0.001	0.005	1.177	0.004	0.003	0.872	0.000	0.001	0.004	•	0.071	0.004
young	0.347	0.021	0.142	0.001	0.148	0.055	0.008	0.000	0.037	0.463		0.001	0.005	0.060	1.904	1.301	0.030	8.534	•	0.023	6.614	0.004	0.061	0.005
stamen	0.277	0.01	0.020	0.044	0.026	0.001	0.004	0.068	0.572	0.521	0.001	0.001	0.005	0.007	1.207	6.233	0.136	28.659	0.000	•	0.021	0.036	0.101	
sepals	0.336	9000	980.0	0.000	0.085	0.007	0.032	0.000	0.076	0.408	•	0.005	0.022	0.007	0.671	1.268	0.015	6.317	0.000	600.0	0.913	0.045	0.196	000'0
petals	9.368	0.001	0.038	0.004	0.037	0.125	0.019	0.000	0.459	0.168	900.0	0.008	0.002	0.011	9.976	1.258	0.203	83.72	000'0	0.020	1.165	0.017	0.034	9000
mature stems	0.029	0.000	0.032	0.001	0.028	0.001	0.000	0.000	0.051	0.636	•	0.007	0.000	0.002	0.492	1.708	0.002	3.644	0000	0.026	26.444	0.034	0.346	0.034
mature leaves	0.53	0.014	0.109	0.001	0.113	990.0	0.012	0.000	0.051	0.541	•		0.007	0.031	1.065	0.627	0.017	4.473	0.000	0.016	9.477	0.053	0.036	0.001
9-11 dpa	1.118	0.267	0.092	0.774	0.110	0.263	2.095	0.097	0.033	1.589	0.017	0.003	0.017	0.332	4.796	0.809	6.983	20.295	0.036	0.123	1.153	0.011	0.031	28.290
6-8 dpa	1.347	0.238	0.116	0.757	0.104	0.228	1.103	0.163	0.032	1.017	0.039	0.005	0.016	0.315	7.361	1.139	4.272		0.015	0.166	0.515	0.014	0.027	17.514
4-5 dpa	0.976	0.183	0.084	0.666	0.095	0.219	1.265	0.131	0.042	1.268	0.028	0.005	0.013	0.283	5.313	0.685	4.301	8.935	0.020	0.161	0.354	0.016	0.014	14.725
2-3 dpa	0.295	090.0	0.057	0.622	990.0	0.112	0.980	0.142	0.045	1.693	0.017	0.008	0.012	0.161	2.152	0.338	3.135	3.812	0.007	0.057	0.210	0.018	0.004	4.947
18-20 dpa	2.477	0.819	0.819	0.419	0.916	0.197	0.960	0.121	0.016	0.749	0.018	0.010	0.024	0.111	10.709	2.670	5.859	20.171	0.080	1.434	14.028	900.0	0.371	3.045
15-17 dpa	2.138	0.735	0.632	0.561	0.732	0.297	0.715	0.163	0.013	0.838	0.001	0.009	0.023	0.131	7.782	2.079	4.398	15.856	0.039	0.892	4.880	0.004	0.167	2.978
12-14 dpa	2.034	0.870	0.511	0.389	0.580	0.362	1.166	0.132	0.021	0.870	0.017	0.009	0.014	0.156	8.460	1.736	3.474	16.012	0.041	0.555	3.455	0.005	0.138	. 0.841
0-1 dpa	0.049	0.040	0.070	0.719	0.075	0.055	0.980	0.163	0.035	1.631	0.001	0.009	0.016	0.114	2.247	0.403	2.555	0.282	0.002	0.011	0.196	0.022	0.005	0.371
-DPA*	0.053*	0.025	0.082	1.313	0.093	0.074	0.276	0.148	0.074	2.989	0.022	0.010	0.016	0.056	1.406	0.095	2.971	1.727	0.000	0.005	0.161	0.024	0.007	0.002
Gene ID/SEQ ID NO.	CT1/1	CT2/2	CT3/3	CT4/4	CT6/5	CT7/6	CT9/7	CT11/8	CT20/9	CT22/10	CT26/11	Ct27/12	CT40/16	CT49/17	CT70/18	CT71/19	CT74/20	CT75/21	CT76/22	CT77723	CT81/24	CT82/25	CT84/27	CT88/13

Reverse-transcription following quantitative PCR was performed using real-time PCR, on tissues of either young or mature cotton (G. hirsutum var Acala) plants. Relative amounts of mRNA of each gene are presented in all examined tissues, dpa- days post anthesis, of ovule and fibers tissues (until 10 dpa) or only fiber tissue (after 10 dpa).

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Two main criteria were used to select cotton genes as candidates that may be involved in fiber development according to their RNA profiling. Genes showing a high degree of fiber expression specificity and genes displaying expression level, which changes concomitantly with fiber development (Table 3, below).

Twenty three genes met these selection criteria:

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CT-1 (SEQ ID NOs. 1 and 106), CT\_2 (SEQ ID NOs.2 and 107), CT\_3 (SEQ ID NOs. 3 and 108), CT\_4 (SEQ ID NOs. 4 and 109) CT\_6 (SEQ ID NOs. 5 and 110), CT\_7 (SEQ ID NOs. 6 and 111), CT\_9 (SEQ ID NOs. 7 and 112), CT\_11 (SEQ ID NOs. 8 and 113), CT\_20 (SEQ ID NOs. 9 and 114), CT\_22 (10 and 115), CT\_26 (SEQ ID NOs. 11 and 116), CT\_27 (SEQ ID NOs. 12 and 117), CT\_40 (SEQ ID NOs. 16 and 118), CT\_49 (SEQ ID NOs. 17 and 119), CT\_70 (SEQ ID NOs. 18 and 120), CT\_71 (SEQ ID NOs. 19 and 121), CT\_74 (SEQ ID NOs.20 and 122), CT\_75 (SEQ ID NOs. 21 and 123), CT\_76 (SEQ ID NOs. 22 and 124), CT\_77 (SEQ ID NOs. 23 and 125), CT\_81 (SEQ ID NOs. 24 and 126), CT\_82 (SEQ ID NOs. 25 and 95), CT\_84 (SEQ ID NOs. 27 and 96) and CT\_88 (SEQ ID NOs. 13 and 26).

CT-4, 22, 20, 27, 40, 82 (SEQ ID NOs: 4, 10, 9, 12, 16 and 25, respectively) were chosen mainly as candidate genes that may have a role in fiber initiation (Table 3) while CT 27 (SEQ ID NO: 12), which is a homologue gene to GL3, was also used as a control (in Figure 2d CT 22, SEQ ID NO: 10 is shown).

CT-1, 2, 3, 6, 7, 9, 49, 70, 71, 74, 75, 76, 77, 81, 84 (SEQ ID NOs. 1, 2, 3, 5, 6, 7, 17, 18, 19, 20, 21, 22, 23, 24 and 27, respectively, see Figures 2a, c) were predicted to be involved in the fiber elongation and quality (strength and finesse) according to their expression pattern (Table 3, Figure 2C CT 1 is shown).

CT11, 40, 74 and CT 26 (SEQ ID NOs. 8, 16, 20 and 11, respectively, see Figures 2a, b) which are homologous to Glabrous1 from Arabidopsis (GenBank Accession No. AB006078) are fiber specific genes that showed uniform and fiber-specific expression during all stages of fiber development (Table 3, in Figure 2B CT 11 is shown as an example). Expression profile of all the chosen genes are shown in Table 2, above.

			-	Table 3		
#15	Gene annotation	Initiation	Fiber Quality & Elongation	Stable and Specific Fiber Expression	Fiber Specific	Biological Process
CT_2	Acid sucrose-6-phosphate hydrolase		>		Yes	carbohydrate metabolism
CT_7	Putative acyltransferase		>			unknown
CT_9	Hypothetical protein		>		Yes	tRNA processing
CT_49	Hypothetical protein		>			unknown
CT_1	GDSL-motif lipase/hydrolase-like protein		^	•		unknown
CT_3	Putative mitochondrial protein		>			unknown
CT_6	Aspartyl protease		. ^			proteolysis and peptidolysis
CT 73	Cysteine protease		>			water deprivation
CT_71	Dehydration-responsive protein		^			dessication
CT_75	Lipid transfer protein, putative					
CT_76	Putative receptor kinase				Yes	protein amino acid phosphorylation
CT_77	Hypothetical protein		. ^		Yes	
CT_81	APETAL2-like protein		۸			cell wall organization and biogenesis
CT_84	Hypothetical protein					aromatic amino acid family biosynthesi
CT 4	Cytochrome P450-like protein	>			Yes	electron transport
CT_20	MYB-related protein homologue	۸	,			regulation of transcription
CT_22	Hypothetical protein	^	•			unknown
CT 27	bHLH transcription factor-like protein	>		•		regulation of transcription
CT 82	MADS box protein-like	>				regulation of transcription
CT_11.	Agamous-like MADS-box transcription factor			۸	Yes	regulation of transcription
CT_26	MYB-related protein homologue			٨	Yes	cell fate commitment
CT_40	Lipid-transfer protein 3 precursor (LTP 3)		•	٨	Yes	lipid transport
CT 74	EN/SPM-like transposon protein			^	Yes	cell wall organization and biogenesis

The selected genes were over-expressed in transgenic arabidopsis and tomato, using the constitutive CaMV promoter of 35S (SEQ ID NO. 31). Transgenic plants were further evaluated for epidermal modifications, trichome density and length and seed hair yield (as further described hereinbelow).

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#### **EXAMPLE 3**

# Analysis of gene expression using publically available microarrays

Further information about the expression of the selected genes (Example 2, above) was retrieved by statistical analysis of microarray data from arabidopsis. Essentially, the best homologs of the new candidate genes in arabidopsis were compared to a set of 77 microarrays experiment of different tissues of Arabidopsis (AtGenExpress databases, the Principal investigator for AFGN: Prof. Dr. Lutz Nover, Botanisches Institut, Molekulare Zellbiologie, FB Biologie und Informatik der J. W. Goethe Universität Frankfurt; Biozentrum N200 3OG, Marie-Curie-Strasse 9, 60439 Frankfurt am Main, <a href="https://www.arabidopsis.org/info/expression/ATGenExpress.jsp">www.arabidopsis.org/info/expression/ATGenExpress.jsp</a>).

Polynucleotide sequences that were highly expressed in elongated cells or inflorescence meristems were selected for further analysis.

Table 4 below lists tissues which exhibit the highest levels of gene expression.

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Table 4

	Tissues with high expression	< Fold change/ specificity	Related to fiber
CT_1	Seed, siliques	10-20	Elongated cells .
CT_11	carpels, flower, seed, siliques	Tissue specific	Flower specific
CT_2	root, seedlin and sepals	Tissue specific	Elongated cells,
CT_22	carpels, flower, inflorescence, shoot	4-10	inflorescence
CT_4	Petals, stamen	>10	Elongated cells,
CT49	siliques .	>2	Elongated cells,
CT_7	carpels, flower, inflorescence, petals, shoot, siliques,	10-30	inflorescence
CT_70	flower, root, stamen	Almost tissue specific	
CT_76	carpels, flower, inflorescence, shoot, siliques	>2	Elongated cells, & inflorescence
CT_77	seeds, pollen, stemen, petals, sepals, siliques	10-50	Elongated cells
CT_82	inflorescence, shoot stem	3-6	inflorescence
CT_88	petals, stamen		Elongated cells

#### **EXAMPLE 4**

# Establishing a correlation between expression of candidate genes and fiber length

In order to define correlations between the levels of RNA expression of the selected genes and fiber length, fibers from 4 different cotton lines were analyzed. These fibers were selected showing very good fiber quality and high lint index (Pima types, originating from other cotton species, namely *G. barbadense*) and different levels of quality and lint indexes from various *G. hirsutum* lines: good quality and high lint index (Acala type), medium lint index (Coker type) and poor quality and short lint index (Tamcot type).

### Experimental procedures

**RNA extraction** - Fiber development stages, representing different fiber characteristic, at 5, 10 15 and 20 DPA were sampled and RNA was extracted as describe in Example 2.

Fiber assessment - Fiber length of the above lines was measured using fibrograph. The fibrograph system was used to compute length in terms of "Upper Half Mean" length. The upper half mean (UHM) is the average length of longer half of the fiber distribution. The fibrograph measures length in span lengths at a given percentage point (www.cottoninc.com/ClassificationofCotton/?Pg=4#Length.)

## Results

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Four different cotton lines were grown in Rehovot, Israel during summer 2004, and their fiber length was measured. The fibers UHM values are summarized in Table 5, below:

25 . *Table 5* 

	Length (UHM)	
Pima S5	. 1.40 ± 0 a	
Acala	1.23 ± 0.01 b	•
Coker 310	1.18 ± 0.01 c	
Tamcot	1.15 ± 0.02 c	

Five genes were tested for correlation between gene expression and fiber length (presented for CT\_76 in Figure 3). The results are summarized in the Table 6 below:

Table 6

Tissue Sampling Day (DPA)    Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Tissue Sampling Day (DPA)   Relative amounts of Relative expression Related to To mRNA   To									
Relative amounts of mRNA mRNA			Tissue Sa	ampling Da	ay (DPA)				
Relative amounts of mRNA   Relative expression Related to form mRNA   Related to mRNA   To mRN		4	0		5	1	0	1	5
CT_1         Coker 310         0.51         4.80         9.3         7.56            Acala         0.64         5.08         7.9         8.01            CT_2         Tamcot         0.03         0.19         7.6         8.17            Coker 310         0.03         0.35         11.4         15.04             Pima S5         0.02         0.36         17.7         15.28             CT_40         Tamcot         0.28          0.47         1.67           Coker 310         0.37          0.47         1.67           Acala         0.30          0.67         2.25           Pima S5         0.37          0.67         2.25           Tamcot         0.01         0.03         5.4         0.01         2.3         0.00         0.10           CT_76         Coker 310         0.01         0.03         5.4         0.01         2.3         0.00         0.10           CT_76         Coker 310         0.01         0.03         5.4         0.01         2.3         0.00         0.10 <td></td> <td></td> <td>amounts</td> <td>amounts of</td> <td>expression Related to</td> <td>amounts of</td> <td>expression Related to</td> <td>amounts of</td> <td>expression Related to</td>			amounts	amounts of	expression Related to	amounts of	expression Related to	amounts of	expression Related to
Acala         0.64         5.08         7.9         8.01           CT_2         Tamcot         0.03         0.19         7.6         8.17           Coker 310         0.03         0.35         11.4         15.04           Acala         0.02         0.36         17.7         15.28           Pima S5         0.02         0.41         23.6         17.58           CT_40         Tamcot         0.28         0.47         1.67           Acala         0.30         0.37         0.46         1.24           Acala         0.30         0.67         2.25           Pima S5         0.37         0.00         0.67         2.25           Tamcot         0.01         0.03         5.4         0.01         2.3         0.00         0.10           CT_76         Coker 310         0.01         0.08         8.9         0.04         5.1         0.00         0.10           Acala         0.01         0.12         16.6         0.06         9.1         0.00         0.12           Pima S5         0.01         0.13         122.4         0.18         177.9         0.12         99.51           Tamco									
CT_26  Tamcot 0.03 0.19 7.6 8.17	CT_1	Coker 310							
CT_2         Coker 310         0.03         0.35         11.4         15.04           Acala         0.02         0.36         17.7         15.28           Pima S5         0.02         0.41         23.6         17.58           CT_40         Tamcot         0.28         0.47         1.67           Coker 310         0.37         0.46         1.24           Acala         0.30         0.67         2.25           Pima S5         0.37         0.067         2.25           Tamcot         0.01         0.03         5.4         0.01         2.3         0.00         0.10           CT_76         Coker 310         0.01         0.08         8.9         0.04         5.1         0.00         0.10           Acala         0.01         0.12         16.6         0.06         9.1         0.00         0.12           Pima S5         0.01         0.13         122.4         0.18         177.9         0.12         99.51           Tamcot         0.50         1.33         2.68         5.03         10.15         1.11         2.24		Acala	0.64	5.08	7.9	8.01			
CT_40  Acala Pima S5  0.02  0.41  23.6  17.58  Tamcot  Coker 310  Acala  0.30  Pima S5  0.37  CT_40  CT_76  Tamcot  0.47  1.67  0.46  1.24  0.67  0.67  0.67  0.25  0.67  0.67  0.25  0.67  0.75  0.67  0.75		Tamcot	0.03	0.19	7.6	8.17			
CT_40	CT 2	Coker 310	0.03	0.35	11.4	15.04			
CT_40         Tamcot Coker 310	U1_2	Acala	0.02	0.36	17.7	15.28			
CT_40		Pima S5	0.02	0.41	23.6	17.58			
CT_40		Tamcot	0.28						
Acaia         0.30         0.07         2.23           Pima S5         0.37         1.03         2.75           CT_76         Tamcot         0.01         0.03         5.4         0.01         2.3         0.00         0.10           Coker 310         0.01         0.08         8.9         0.04         5.1         0.00         0.10           Acaia         0.01         0.12         16.6         0.06         9.1         0.00         0.12           Pima S5         0.01         0.13         122.4         0.18         177.9         0.12         99.51           Tamcot         0.50         1.33         2.68         5.03         10.15         1.11         2.24	CT 40	Coker 310	0.37						
CT_76	01_40	Acala	0.30						
CT_76		Pima S5	0.37					1.03	
CT_76         Acala         0.01         0.12         16.6         0.06         9.1         0.00         0.12           Pima S5         0.01         0.13         122.4         0.18         177.9         0.12         99.51           Tamcot         0.50         1.33         2.68         5.03         10.15         1.11         2.24		Tamcot	0.01	0.03	5.4	0.01	2.3	0.00	
Acaia         0.01         0.12         16.6         0.06         9.1         0.00         0.12           Pima S5         0.01         0.13         122.4         0.18         177.9         0.12         99.51           Tamcot         0.50         1.33         2.68         5.03         10.15         1.11         2.24	CT 76	Coker 310	0.01	0.08	8.9	0.04	5.1	0.00	
Tamcot 0.50 1.33 2.68 5.03 10.15 1.11 2.24	0/0	Acala	0.01	0.12	16.6				
		Pima S5	0.01	0.13	122.4	0.18	177.9	0.12	99.51
CT 91 Color 240 0 024 2 24 9 65 4 51 14 76 0 94 2 75		Tamcot	0.50	1.33	2.68	5.03			
	CT_81	Coker 310	0.31	2.64	8.65	4.51	14.76	0.84	2.75
Acala         0.49         4.38         8.98         6.36         13.05         3.65         7.49		Acala	0.49	4.38	8.98	6.36			

Reverse-transcription following quantitative PCR was performed using real-time PCR, on tissues of 0, 5 10 and 15 DPA of cotton (G. hirsutum var Tamcot, Coker and Acala, and G. barbadense var Pima S5) plants. Relative amounts of mRNA and Relative expression related to T0 of each gene are presented in all examined tissues.

#### EXAMPLE 5

# Cloning of the selected genes in a binary vector under constitutive regulation and recombinant expression of the same

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ORF analysis - Gene sequences of the present invention were analyzed for ORFs using Gene Runner software version 3.05 (Hasting Software, Inc: <a href="https://www.generunner.com/">www.generunner.com/</a>). ORFs of each gene were compared to Genbank database, using Blast (<a href="https://www.ncbi.nlm.nih.gov/BLAST/">www.ncbi.nlm.nih.gov/BLAST/</a>). By comparing to highest homologous ORFs, the position of the ATG initiation codon was determined. All the sequences described herein were shown to have a predicted full length ORF and to include the predicted ATG starting codon.

Cloning into the pPI expression vector - For cloning genes of the present invention, total RNAs from the various developmental stages of fiber producing cells was extracted, using Hot Borate RNA Extraction from Cotton Tissue according to <a href="https://www.eeob.iastate.edu/faculty/WendelJ/rnaextraction.html">www.eeob.iastate.edu/faculty/WendelJ/rnaextraction.html</a>. Complementary DNA (cDNA) molecules were produced from mRNA using M-MuLV reverse-transcriptase (RT) enzyme (Roche) and T<sub>16</sub>NN DNA primer, following protocol provided by the

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manufacturer. cDNA amplification was done for 19 genes, out of the sequences above, namely CT clones number 1, 2, 3, 6, 7, 9, 11, 20, 22, 27, 40, 71, 74, 75, 76, 81, 82, 84 and 88, by PCR using PFU proof reading DNA polymerase enzyme (Promega www.promega.com/pnotes/68/7381\_07/7381\_07.html) following the protocol provided by the manufacturer. Primers for each gene were designed to span the full ORF. Additional restriction endonuclease sites were added to the 5' end of each primer to facilitate further cloning of the CTs to the binary vector (pPI). Table 7 below, lists the primers used for cloning each of the genes:

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	Forward	Reverse	upstream restriction	downstream restriction site
No	Primer/SEQ ID NO:	Primer/SEQ ID NO:	site	
CT_1	ACCCGGGATGGTTATTGTAGCAGAAGG/32	GCCGAGCTCGAATCAATGAGGGCAATGCC/33	Smal	SacI
CT_2	AATCTAGACAAGTACAGAAGCTCAATTCCC/34	TGATAATCATGTGGAAGCAACC/35	XbaI	
CT_3	CAGCCCGGGTGATGGAACTGAGCATTCAG/36	CGTGAGCTCTGATTAGAGTTTCAAGTGCATG/37	Smal	SacI
CI_6	TITCCCGGGTTGTTGTCATGGCTTCTCTGC/38	ATGGAGCTCATATTCATGGCCAAAACAC/39	Smal	SacI
CT_7	G CACCCGGGAAAGGAAATGGCAGGCGTC/40	TITCGATATCCACAGTACCCTACTTCCATGC/41	Smal	ECORV
6 L	TACCCGGGTACCATTACTCTACTACAGCTGC/42	GAGAGCTCAACAGACAAGACCAGACTGG/43	SmaI	Saci
CT_11	ACCCCGGGCAAGTCAAAGAGAATGG/44	CATGAGCTCTTCTCCAACTCCTCTACCC/45	SmaI	SacI
CT_20	CCCCCGGGTCCCTATTGCATGCCTTTC/46	TTGAGCTCACTCGATCTTACTCATCC/47	Smal	SacI
CT_22	AGCCCGGGAGATAGAGATGGGAGGTCC/48	TCGAGCTCTGGGGCAACATCATTTACC/49	Smal	SacI
CT_27	rccccccccrcarcraarrcracrcc/50	TTGGATATCGCACCTTATGACATGGGATC/51	SmaI	ECORV
CT_40	TTCCCGGGTACAACATGGCTAGTTCCG/52	TCGAGCTCATCAACCTCACTGCACCTTG/53	SmaI	SacI
CT_71	TAGTCACTCCTGTTCTAGATGAAG/54	CTGAGCTCCAGGATTTTTACTTAGGGACCC/55	XbaI	SacI
CT_74	TACCCGGGCATACAGAGATGGAGAGGC/56	ACGAGCICAAAGGIGITIGCITAGGICC/57	SmaI	SacI
CT_75	AGCCCGGGAGAAAGATGAAAAGGGG/58	AAGATATCAAATCCCATGCAAAACCCC/59	Smal	ECORV
	AACCCGGGCGGCACTTAAAAGAAAACC/60	AAGAGCTCCTTTGTTGGCTTCTCAAG/61	Smal	Saci
CT_81	GACCCGGGACTGTAAAAAGCATAGG/62	GCGAGCTCAGCTTAAGGATGATGGGGAG/63	SmaI	SacI
CT_82	ATCCCGGGGATGGTGAGGCCAAAATTC/64	ACGAGCTCTAGCAATGGCGATAACGTAC/65	SmaI	SacI
CT_84	ATCCCGGGTTCCATGAAAAGGGTCTCG/66	GTGAGCTCTATCGTCGTTGTCCTTCAGC/67	SmaI	SacI

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The resultant PCR blunt ended products, were purified using PCR Purification Kit (Qiagen, Germany), digested with the appropriate restriction endonucleases (Roche) and cloned into the pPI binary vector (Figure 4), while replacing the existing GUS reporter gene. pPI is a modified version of pBI101.3 (Clontech, Accession No. U12640). pPI was constructed by inserting a synthetic poly-(A) signal sequence, which originated from pGL3 Basic plasmid vector (Promega, Acc No U47295, where the synthetic poly-(A) signal sequence is located between base-pairs 4658-4811), into the HindIII restriction site of pBI101.3 (while reconstituting the HindIII site, downstream to the poly-(A) insert), to avoid the possibility of read-through effect of the upstream Nos-promoter. To replace the GUS gene with each one of the CT genes in the pPI binary vector, pPI was digested with the appropriate restriction enzymes [5' prime restriction enzyme is either Smal or Xbal and 3' prime restriction enzyme is either SacI or EcoRV (Roche- using the protocol provided by the manufacturer)]. Open binary vector was purified using PCR Purification Kit (Qiagen, Germany). 5-75 ng of PCR product of each of the CT genes and 100 ng of open pPI plasmid vector were ligated in 10 µL ligation reaction volume using T4 DNA ligase enzyme (Roche), following the protocol provided by the manufacturer. Ligation products were introduced into E. coli cells.

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Recombinant expression in bacteria - 60 μL of E. coli, strain DH5-α competent cells (about 10<sup>9</sup> cells/mL) were transformed using 1μl of ligation reaction mixture by electroporation, using a MicroPulser electroporator (Biorad), 0.2 cm cuvettes (Biorad) and EC-2 electroporation program (Biorad). E. coli cells were grown on 0.8 mL LB liquid medium at 37 °C for 1 hrs and 0.2 mL of the cell suspension were plated on LB-agar plates supplemented with the antibiotics kanamycin 50 mg/L (Sigma). Plates were then incubated at 37 °C for 16 hrs. Bacteria colonies were grown and expression was confirmed by PCR amplification using primers which were designed to span the inserted sequence in the binary vector. Primers used for DNA amplification of the inserts in the pPI binary vector were: 5'-GGTGGCTCCTACAAATGCCATC-3' (forward, SEQ ID NO. 70) and 5'-AAGTTGGGTAACGCCAGGGT-3' (reverse, SEQ ID NO. 71).

PCR products were separated on 1.5 % agarose gels and product sizes were estimated by comparing to DNA ladder (MBI Fermentas). PCR products with the

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predicted size were sequenced using the same primers previously used for PCR amplification (See Table 7, above).

Additional primers, which were designed based on the sequence of each gene insert, were used to complete the sequencing of the full length ORF insert.

Sequencing of the inserted sequence was performed to verify that the clones were introduced in the right orientation, and to eliminate the possibility that sequence errors were included during PCR amplification. DNA sequences were determined using ABI 377 sequencer (Amersham Biosciences Inc).

Into each one of the 19 pPI binary constructs harboring the CT genes, the constitutive, Cauliflower Mosaic Virus 35S promoter was cloned.

Cauliflower Mosaic Virus 35S promoter sequence, originated from the pBI121 vector (Clontech, Accession No AF485783) was cloned by digesting the pBI121 vector with the restriction endonucleases HindIII and BamHI (Roche) and ligated into the binary constructs, digested with the same enzymes (SEQ ID NO. 31).

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#### EXAMPLE 6

# Agrobacterium transformation of binary plasmids harboring the genes of interest and expression in Arabidopsis and tomato plants

Each of the nineteen binary constructs, comprising the 35S promoter upstream of each of the CTs genes was transformed into Arabidopsis or tomato plants via Agrobacterium tumefacience transformation.

60 μL of Agrobacterium tumefaciens GV301 or LB4404 competent cells (about 10<sup>9</sup> cells/mL) were transformed with 20 ng of binary plasmid via electroporation, using a MicroPulser electroporator (Biorad), 0.2 cm cuvettes (Biorad) and EC-2 electroporation program (Biorad).

Agrobacterium cells were grown on 0.8 mL LB liquid medium at 28 °C for 3 hrs and 0.2 mL of the cell suspension were plated on LB-agar plates supplemented with the antibiotics gentamycin 50 mg/L (for Agrobacterium strains GV301) or streptomycin 300 mg/L (for Agrobacterium strain LB4404) and kanamycin 50 mg/L (Sigma). Plates were then incubated at 28 °C for 48 hrs. Agrobacterium colonies were grown and PCR amplification was performed on Agrobacterium cells, using primers which were designed to span the inserted sequence in the binary vector.

Primers used for PCR amplification were: 5'

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GGTGGCTCCTACAAATGCCATC-3' (forward, SEQ ID NO. 70) and 5'-AAGTTGGGTAACGCCAGGGT-3' (reverse, SEQ ID NO. 71).

PCR products were separated on 1.5 % agarose gels and product sizes were determined by comparing to DNA ladder (MBI Fermentas). PCR products with the predicted size were sequenced using the primers which were used for the PCR amplification. Sequencing of the inserted sequence was performed to verify that the right clones were introduced into the Agrobacterium cells.

DNA sequencing was effected using ABI 377 sequencer (Amersham Biosciences Inc.).

# Plant transformation and cultivation:

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Transformation of Arabidopsis thaliana plants with putative cotton genes -Arabidopsis thaliana Columbia plants (T0 plants) were transformed using the Floral Dip procedure described by Clough and Bent and by Desfeux et al., with minor modifications. Briefly, T0 Plants were sown in 250 ml pots filled with wet peat-based growth mix. The pots were covered with aluminum foil and a plastic dome, kept at 4 °C for 3-4 days, then uncovered and incubated in a growth chamber at 18-24 °C under 16/8 hr light/dark cycles. The T0 plants were ready for transformation six days prior to anthesis. Single colonies of Agrobacterium carrying the binary constructs, were cultured in LB medium supplemented with kanamycin (50 mg/L) and gentamycin (50 mg/L). The cultures were incubated at 28 °C for 48 hrs under vigorous shaking and then centrifuged at 4,000 rpm for 5 minutes. The pellets comprising Agrobacterium cells were re-suspended in a transformation medium. containing half-strength (2.15 g/L) Murashig-Skoog (Duchefa); 0.044 µM benzylamino purine (Sigma); 112 µg/L B5 Gambourg vitamins (Sigma); 5 % sucrose; and 0.2 ml/L Silwet L-77 (OSI Specialists, CT) in double-distilled water, at pH of 5.7. Transformation of T0 plants was effected by inverting each plant into an Agrobacterium suspension, such that the above ground plant tissue was submerged for 3-5 seconds. Each inoculated T0 plant was immediately placed in a plastic tray, then covered with clear plastic dome to maintain humidity and was kept in the dark at room temperature for 18 hrs, to facilitate infection and transformation. Transformed (i.e., transgenic) plants were then uncovered and transferred to a greenhouse for recovery and maturation.

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The transgenic T0 plants were grown in the greenhouse for 3-5 weeks until siliques were brown and dry. Seeds were harvested from plants and kept at room temperature until sowing. For generating T1 transgenic plants harboring the genes, seeds collected from transgenic T0 plants were surface-sterilized by soaking in 70% ethanol for 1 minute, followed by soaking in 5% sodium hypochloride and 0.05% triton for 5 minutes. The surface-sterilized seeds were thoroughly washed in sterile distilled water then placed on culture plates containing half-strength Murashig-Skoog (Duchefa); 2 % sucrose; 0.8 % plant agar; 50 mM kanamycin; and 200 mM carbenicylin (Duchefa). The culture plates were incubated at 4 °C for 48 hours then transferred to a growth room at 25 °C for an additional week of incubation. Vital T1 Arabidopsis plants were transferred to a fresh culture plates for another week of incubation. Following incubation the T1 plants were removed from culture plates and planted in growth mix contained in 250 ml pots. The transgenic plants were allowed to grow in a greenhouse to maturity.

Transformation of Micro-Tom tomato plants with putative cotton genes - Tomato (Lycopersicon esculentum, var MicroTom) transformation and cultivation of transgenic plants was effected according to Curtis et al. 1995, and Meissner et. al. 2000.

20 *EXAMPLE* 7

## Growth of Arabidopsis transformed plants and phenotype characterizations

T1 arabidopsis plants were grown as described above and phenotypes were characterized.

PCR analysis of transgenic plants - Arabidopsis T2 seeds were sown directly in growth mix contained in 250 ml pots. Positive transgenic plants were screen for kanamycin resistance in two weeks old leaves by PCR. Primers used for PCR amplification of the kanamycin were: 5'- CTATTCGGCTATGACTGGGC -3' (forward, SEQ ID NO. 72) and 5'- ATGTCCTGATAGCGGTCCGC -3' (reverse, SEQ ID NO. 73).

Root performance - In order to visualized root performance, T2 seeds were surface-sterilized by soaking in 70 % ethanol for 1 minute, followed by soaking in 5 % sodium hypochloride and 0.05 % triton for 5 minutes. The surface-sterilized seeds were thoroughly washed in sterile distilled water and then placed in culture plates

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containing half-strength Murashig-Skoog (Duchefa); 2% sucrose; 0.8% plant agar; 50 mM kanamycin; and 200 mM carbenicylin (Duchefa). The culture plates were incubated at 4 °C for 48 hours then transferred to a growth room at 25 °C till reaching the right size for phenotypic characterization.

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Results

Table 8 - Analysis of Arabidopsis T2 plants caring the putative cotton genes

CT	Putative Gene function	T generation	No of Independent plants	T2 Phenotype
CT_11	Agamous-like MADS-box transcription factor	2		Curled and narrow leaves, with long petioles, roots are longer and denser (Figures 5a-c)
CT.9	· Hypothetical protein	7	<b>.</b>	The rosette leaves and the inflorescent are longer and bigger compared to control. The roots are longer and denser.  The phenotype resembles the phenotype of Arabidopsis plants over expressing expansin as was characterized by Hyung-Taeg Cho and Daniel J. Cosgrove in PNAS u August 15, 2000.  (Figures 5g-i)
CT_20	MYB-related protein	-	-	Small rankled and hairy leaves (Figures 5d and e)
CT_40	Lipid-transfer protein 3	7		Longer and curlier leaves (Figure 5j)
CT_22	Hypothetical protein			Narrow leaves, with long petioles (Figures 5d and f)
CT_81	APETAL2-like protein	1	. 1	The rosette leaves are almost double then wild type (Figures 5k and l)
CT_1	hydrolase-like protein	1	9 :	Narrow leaves, with long petioles (same as CT_22, not shown)

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# EXAMPLE 8

Growth of MicroTom transformed plants and phenotype characterizations

Experimental Procedures

Transgenic tomato plants - Plant were transformed as described in Example 6, above. Following transformation, T1 MicroTom tomato plants were grown in mix contained in 1000 ml pots.

Table 9 - Analyzing Micro-Tom tomato T1 and T2 plants and seeds caring the putative cotton genes Results

CT	Putative Gene function	T generation	No of Independent plants	TI seed hair length (wt 0.3mm)	T2 Phenotype
CT20	MYB-related protein homologue	I	10	0.366±0.006mm (Figures 6c-e)	Small and wrinkled leaves, the trichome on the leaves are longer and denser. (Figure 6a-b)
CT75	Lipid transfer protein, putative	· I	2	0.347±0.019mm	Big inflorescent
cr_6	Aspartyl protease	. 1	1	0.343±0.019	
CT_82	MADS box protein-like	1	£	0.423±0.013mm (Figure 5f)	Normal plants

# 54 **Discussion**

# (Examples 1-8)

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In-silico identification of genes involved in cotton fiber development - Little is known about the genetic control of cotton fiber initiation and elongation. Since both cotton fiber and Arabidopsis trichomes are developed from single epidermal cells they are assumed to share similar genetic regulation (Reviewed at Wagner G.J. et. al. 2004). In Arabidopsis, a large number of studies have revealed extensive information on the genetic mechanisms regulating trichome initiation and elongation. Several studies demonstrated the similarities between trichome and fiber by showing that cotton fiber specific promoters in arabidopsis and tobacco plants confer trichome specific expression (Kim and Triplett, 2001; Hsu et. al. 1999; Liu et. al. 2000, Wang et al. 2004). Most of the research that studies fiber development uses arabidopsis trichome as a model system to identify cotton genes in a small scale manner (Kim and Triplett, 2001; Wang et al. 2004).

In this study the present inventors have used tomato trichome and flower EST libraries as model systems to study cotton fiber development. Analysis of the EST libraries profile of the tomato homologous clusters to known arabidopsis trichome genes showed that tomato trichome and flower EST libraries significantly contributed to this set of clusters.

This result was confirmed while analyzing the EST libraries profile of the new cotton clusters that were selected by their RNA expression pattern as cotton fiber genes. 9 and 10 clusters contained ESTs which originated from the flower and trichome libraries respectively. Furthermore the group of tomato trichome clusters (trichome ESTs/total ESTs> 0.1) comprise large portion from the tomato genes that show high degree of homology to cotton (~ 50 %) even though their percentage in the total population is only ~5 %. It may indicate that both organ share common developmental processes. Even though there is a large group of studies about the genetic control of tomato fruit and trichome development no publications could be found to use these organs as a source of genomic data to study cotton fiber development. All of the 23 cotton genes were compared to unique EST data produced separately from embryo and suspensor of Scarlet Runner bean developing seeds (www.mcdb.ucla.edu/Research/Goldberg/ests/intro-index.htm). All sequences, except one, share high homologies with sequences originated from the suspensor, which is a

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maternal tissue. This result supports the *in silico* results and identifies the role of these cotton clusters in fiber development, which originated from maternal cells as well.

Identifying cotton genes with a role in fiber development through analysis of RNA expression profile - The differentiation/initiation phase is represented by gene expression at or before anthesis. The elongation phase mainly in hirsutum cultivars is represented by very fast growth rate mainly during 5 to 20 DPA. One pattern is represented by genes such as CT 1, 2, 3 expressed at their highest levels, slightly before and during the period of peak fiber expansion about 20 DPA. Another pattern of gene expression is displayed by the CT40, 11 or 70 which have the same expression level throughout all fiber development. Likewise, known genes encoding actin, endoxyloglucan transferase or Suc synthase also display unvarying RNA levels throughout fiber development (Shimizu et al., 1997).

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Since the initiation occurs mainly before anthesis till 1 DPA it suggests that genes with a peak in expression during this time may have a role in fiber initiation. CT 4, 20, 22 and 11 have expression patterns that indicate their involvement at this stage.

One limitation of the current cotton EST database is the absence of ESTs that were extracted from flower at initiation stage (there is one library that was taken from ovary 1 DPA but of poor quality) most ESTs were taken only later on, between 6 to 10 DPA. This EST composition could explain why most of the chosen genes have expression pattern that indicate their association with the elongation stage.

Role of the selected genes in fiber development, possible mechanisms - The 23 fiber-associated clusters could be classified into 6 functional categories according to their sequence homology to known proteins and enzymes (Table 3, above). The classification was made according to the GO consortium (www.geneontology.org/). The largest group comprises unique sequences without homology to any known protein. The rest of the clusters were classified according to categories known to be associated with fiber development. Two genes (Table 3, above) were classified into a cell fate commitment category: a new gene that belongs to the MYB transcription factor and a cotton homologous gene to GL3 that are known to be involved in trichome development in arabidopsis. The expression pattern of both genes and the phenotype of CT20 transgene both in arabidopsis and tomato T1 plants support their involvement mainly in the initiation phase.

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Accumulative evidence link cotton MYB genes with fiber development (Suo. J. et. al. 2003, Cerdoni. M.L. et. al. 2003, Loguerico L.L. et al 1999). Over expression of a number of genes that work in the same pathway related to the initiation phase, could further induce initiation. Kirik et al. (2004) showed that by over-expressing two or three genes from the initiation phase they enhance the number of trichome and root hairs. Genes that relate to the initiation phase could be used for uniformity of fiber initiation on the cotton seed, initiate of more of the seeds epidermis cells into fibers. Over expression of those genes in vegetative meristems such as stems and leaves could be used as protect against insects (as has been shown in canola, www.westerngrains.com/news/nr\_050413.html) and a-biotic stresses. However, there is no substantial evidence that proves direct involvement of any MYB gene to fiber development.

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Two other genes (Table 3, above) are transcription factors from the MYB and MADS BOX families. Many studies demonstrated the function of these two transcription factor families as homeotic genes with key role in different developmental processes, among them are trichome and fiber morphogenesis (Suo. J. et. al. 2003, Ferrario S et. al. 2004). Their role in early stages of fiber development is supported also by their RNA expression pattern, which, is induced before, and during the day of anthesis. One gene (CT\_2, Table 3, above) was classified to the pathways of starch and sucrose metabolism. A recent work demonstrates that another gene (SUS), which, belongs to this pathway, is a limiting factor in both fiber initiation and development. CT 40, 75 were classified as lipid transport whose RNA expression is highly induced during early fiber elongation stage fit to the fact that lipids are key components in fiber formation. Several genes (Table 3, above, CT\_4, 70, 71) were classified either as genes involved in desiccation, salinity response stimulated by abscisic acid and genes involved in electron transfer. Out of them 3 genes (CT 7, 9 and 49) were selected by RNA expression pattern to be induced in the elongation stage. Several studies consider changing proton and potassium pump mechanisms as key factor in the rapid growth rate of the fiber (Smart L.B, et. al. 1998). Combine the over-expression of several genes relate to fiber elongation such as genes relate to starch and sucrose metabolism that will enhance cell wall formation with lipid transport genes or genes relate to desiccation that my influence on the pressure in the cell, might result in longer fibers then over expressed of single gene.

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#### EXAMPLE 9

# Cloning and analyses of promoter sequences upstream of the genes of the present invention

Differential gene expression in fiber tissues vs. other tissues in cotton is the result of complicated gene regulation. The genomic regions upstream of the 23 selected genes are predicted to possess promoter activities that direct gene expression to fiber cells in unique quantitative and qualitative manner. A precise gene expression, directed to fiber cells, is crucial for the development of cotton plants with enhanced fiber performance, without negatively affecting other plant tissues.

### **Experimental Procedures**

Cloning of promoter sequences - The genomic sequence upstream of CT2 and CT6 were cloned from genomic DNA of cotton (Gossypium hirsutum L. var Acala), as follows. Total genomic DNA was extracted from plant leaf tissues of 4 week old cultivated cotton plants (Gossypium hirsutum L., var Acala), using DNA extraction kit (Dneasy plant mini kit, Qiagen, Germany). Inverse PCR (IPCR), DNA digestion, self-ligation, and PCR reaction were performed on genomic DNA, following common protocol (www.pmci.unimelb.edu.au/core facilities/manual/mb390.asp) with the following modifications. To avoid mistakes in the IPCR, the genomic sequence of the 5' sequence of a relevant cDNA (i.e. including introns) was first identified to produce Genomic Island (GI). The desired region from the genomic DNA was PCR-amplified using direct oligonucleotide primers designed based on the cDNA cluster sequence (for CT\_2 and CT\_6, respectively GI sequences are as set forth in SEQ ID NOs. 74 and 75 for CT\_2 and CT\_6. Primers are set forth in SEQ ID NOs. 14-15 (CT\_2) and 101-102 CT\_6). PCR reaction was performed in a DNA thermal cycler, using common PCR protocols. For example:

92 °C/3 min  $\rightarrow$  31 × [94 °C/30 sec  $\rightarrow$  56 °C/30 sec  $\rightarrow$  72 °C/3 min]  $\rightarrow$  72 °C/10 min).

PCR products were purified using PCR purification kit (Qiagen) and sequencing of the amplified PCR products was performed, using ABI 377 sequencer (Amersham Biosciences Inc).

In some cases, a different technique [UP-PCR (Dominguez and Lopez-Larrea. 1994)] was used when IPCR resulted in poor amplification. UP-PCR technique was

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SMs were placed in a thermocycler (Biometra, USA), where it was subjected to an amplification program of 1 minute at 90 °C, held (pause) at 80 °C until PM was added, 30 seconds at 15 °C, 10 minutes at 25 °C, 3 minutes at 68 °C, held at 90 °C until the external SP (2 µl of 10 µM concentration) was added. The process was followed by external PCR reaction of 30 seconds at 92 °C, 10 seconds at 94 °C, 30 seconds at 65.5° C, 3 minutes at 68 °C, for 30 cycles followed by final extension of 10 minutes at 68 °C.

External PCR product diluted 5000 – 25000 fold was used as a template, and PCR amplification was effected using specific internal sWP and SP (30 pmol each) primers, 1U Ex Taq (Takara), in 50µl reaction volume. Internal PCR reaction was subjected to an amplification program of 2 minutes at 92 °C, followed by 30 seconds at 94 °C, 30 seconds at 58 °C, and 3 minutes at 72 °C for 30 cycles and a final extension of 10 minutes at 72 °C. IPCR / Up-PCR products were purified (PCR Purification Kit, Qiagen, Germany) and sequenced (ABI 377 sequencer, Amersham Biosciences Inc).

Primers for CT 2 were as follows (UP-PCR):

External primers:

sWP-5'-TTTTTGTTTGTTGTGGG-3' (SEQ ID NO: 80)

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SP (Internal)- 5'- GCTCCGGGCTTTGGTTAACG -3' (SEQ ID NO: 81)

Internal genomic sequence of CT\_2 resulting from the above procedure is provided in SEQ ID NO: 14.

Primers for CT\_6 were as follows (UP-PCR):

External primers:

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sWP-5'-TTTTTGTTTGTTGTGGG-3' (SEQ ID NO: 83)

SP (Internal)- 5'- GGTGGTGGGCTCTTGCAACAG -3' (SEQ ID NO: 84)

Internal genomic sequence of CT\_2 resulting from the above procedure is provided in SEQ ID NO: 85.

For cloning the putative promoters and 5' UTRs, PCR amplification was carried out using a new set of primers (below) to which 8-12 bp extension that included one restriction site (*Hind*III, *Sal*I, *Xba*I, *Bam*HI, or *Sma*I) on the 5' prime end. For each promoter, restriction sites that do not exist in the promoter sequence were selected. Moreover, the restriction sites in the primer sequences were design so the resultant PCR products will be cloned into the binary vector pPI in the right orientation, upstream of the GUS reporter gene.

The plasmid pPI was constructed by inserting a synthetic poly-(A) signal sequence, originating from pGL3 basic plasmid vector (Promega, Acc No U47295; bp 4658-4811) into the *Hind*III restriction site of the binary vector pBI101.3 (Clontech, Accession No. U12640).

Below are the primers used for promoter and 5' UTR (P+U) amplification and cloning into pPI, and the amplified and cloned sequence. Restriction sites within each primer are shown in bold letters:

CT 2:

P+U forward (HindIII): 5'- ATTCAAGCTTTTTTTGTTTGTGGGGG - 3' (SEQ ID NO: 86)

P+U reverse (BamHI): 5'- TTGGATCCTTGGGCATTGAGCTTCTGTAC - 3' (SEQ ID NO: 87)

P+U sequence of CT\_2 is as set forth in SEQ ID NO: 88. CT6:

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P+U forward (HindIII): 5'- TTAAAGCTTTGGGCTCTTGCAACAGAGGC - 3' (SEQ ID NO: 89)

P+U reverse (BamHI): 5'- AAGGATCCGACGACAACAACAACAACAAC -3' (SEQ ID NO: 90)

P+U sequence of CT\_6 is as set forth in SEQ ID NO: 91.

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Genomic DNA or the IPCR/UP-PCR product was used as DNA template for PCR-amplification, using the newly designed oligonucleotide primers. PCR products were purified (PCR Purification Kit, Qiagen, Germany) and digested with the restriction sites exist in the primers (Roche, Switzerland). The digested PCR products were re-purified and cloned into the binary vector pPI, which was digested with the same restriction enzymes. PCR product and the open plasmid vector were ligated using T4 DNA ligase enzyme (Roche, Switzerland).

#### EXAMPLE 10

# Transforming Agrobacterium tumefacience cells with binary vectors harboring cotton fiber promoters

pPi Binary vector, including either CT2 or CT6 promoter, upstream to the GUS reporter gene were used to transform *Agrobacterium* cells.

The binary vectors were introduced to Agrobacterium tumefaciens GV301, or LB4404 competent cells (about 10<sup>9</sup> cells/mL) by electroporation. Electroporation was performed using a MicroPulser electroporator (Biorad), 0.2 cm cuvettes (Biorad) and EC-2 electroporation program (Biorad). The treated cells were cultured in LB liquid medium at 28°C for 3 hr, then plated over LB agar supplemented with gentamycin (50 mg/L; for Agrobacterium strains GV301) or streptomycin (300 mg/L; for Agrobacterium strain LB4404) and kanamycin (50 mg/L) at 28°C for 48 hrs. Agrobacterium colonies which developed on the selective media were analyzed by PCR using the primers set forth in SEQ ID NOs: 70-71, which were designed to span the inserted sequence in the pPI plasmid. The resulting PCR products were isolated and sequenced as described in Example 4 above, to verify that the correct sequences were properly introduced to the Agrobacterium cells.

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# Cotton fiber specific promoters are expressed in tomato leaves and tomato fruits

GUS staining was effected to illustrate specific expression in trichomes and tomato fruits.

# **Experimental Procedures**

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Transformation of Micro-Tom tomato plants with putative cotton promoter - As describe above.

Transformation of Arabidopsis thaliana plants with putative cotton promoter - As describe above.

GUS staining of Arabidopsis- Gus staining of arabidopsis plants was effected as previously described (Jefferson RA. et. al. 1987, Meissner et. al. 2000).

GUS staining of tomato leaves - Gus staining of tomato plants was effected as previously described (Jefferson RA. et. al. 1987, Meissner et. al. 2000).

Tissue fixation was effected as follows. Tomato leaves were immersed in 90 % ice cold acetone, then incubated on ice for 15 - 20 minutes following by removal of the acetone. Thereafter tissue was rinsed twice with the Working Solution [100 mM Sodium Phosphate (Sigma, USA) buffer pH=7, Ferricyanide (Sigma, USA) 5 mM, Ferrocyanide (Sigma, USA) 5 mM, EDTA (BioLab) pH=8 1 mM, Triton X-100 (Sigma, USA) 1 %] for 15-20 minutes in dark. Rinsing solution was then removed and replaced with X-gluc staining solution [Working Solution + 5-bromo-4-chloro-3indolyl-β-D-glucuronic acid (X-GlcA. Duchefa) solubilized Dimethylformamide (BioLab) 0.75mg/ml, Dithiothreitol (BioLab) 100mMl and incubated for over night at 37 °C in the dark (tubes wrapped with aluminum foil). Distaining was effected by sinking the plant tissue in 70 % ethanol and heating at 50. °C for ~120 minutes. Distaining step was repeated until the plant tissue became transparent excluding the blue stained regions. Distained plants were stored in 70 % ethanol (BioLab) at room temperature.

GAS staining of Tomato Fruits - Gus staining of tomato fruits was effected as previously described (Jefferson RA. et. al. 1987, Meissner et. al. 2000). Briefly: thin tomato fruit slice were sunk in staining solution [100 mM Sodium Phosphate (Sigma, USA) buffer pH=8, Ferricyanide (Sigma, USA) 5 mM, Ferrocyanide (Sigma, USA) 5 mM, EDTA (BioLab) pH=8 15mM, Methanol (BioLab) 20 %, 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-GlcA, Duchefa) solubilized in N,N-Dimethylformamide (BioLab) 0.75mg/ml] in the dark (tubes wrapped with aluminum

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foil) and incubated for over night at 37 °C. Distaining was effected by sinking the plant tissue in 70 % ethanol and heating to 50 °C for ~20 minutes. Distaining step was repeated until the fruit slice became transparent except for the blue stained regions. Distained fruits were stored in 70 % ethanol (BioLab) at room temperature.

#### Results

# GUS staining was performed on Seeds of T1 tomato plants.

GUS was expressed under the regulation of CT2 and CT6, promoters in the genetically transformed tomato plants (Figures 7a-b).

Results for tomato T1 generation are summarized in the Table 10, below.

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Table 10

Promoter	No of Independent T1 plants	Leaf	Leaf trichome	Seed cover of Young fruit	Seed cover of Mature green	Seed cover of Ripen fruit
CT2	four	0	2	3	5	3
СТ6	one	0	1	1	2.5	1

The numbers represent average grade, 0-not expressed, 5-high expression

#### **EXAMPLE 12**

# Tomato seed hairs as a model system for cotton fibers

The genetic modification of cotton is long and time consuming. Hence to find genes which are capable of improving cotton fiber yield and quality, a need exists for a model system for cotton fiber development in other plants.

Trichome cells and root hairs share common characteristics with cotton fiber cells, and are widely accepted as model systems for cotton fiber development [Reviewed in Wagner. G.J. et. al. 2004) and Wang et al. 2004].

However measuring changes in growth rate, length and thickness as well as other structural parameters is not an easy task because of the small size, remote accessibility and lack of uniformity in sizes of trichome cells.

To overcome these limitations, tomato seed hairs were analyzed for their possible use as a model tissue for cotton fiber development. To this end, the GUS reporter gene was over-expressed under the regulation of cotton fiber specific promoter element derived from CT2, as describe above.

Tomato transformation of the binary construct, plant regeneration and GUS staining was effected as described above.

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Tomato seed hairs (Figure 8a) are maternal epidermal cells, covering the ovule surface of the seeds. In anatomical aspects, tomato seed hairs are much closer to cotton fibers than either trichome cells or root hairs.

4 independent transgenic tomato fruits over-expressing GUS gene under cotton specific promoter CT\_2 were produced. GUS staining of fruits at the mature-green stage (fruit is in full size just before the ripening process) was observed uniquely on the seed envelope, where seed hairs are being developed (Figures 7a and b).

Five independent transgenic tomato fruits over-expressing 35S-expansin (AF043284) were produced, and the seed hair length was measured and compare to wt. The seed hair of transgenic plants was significantly longer than of wt (Figures 8a-b).

Table 11

Plant	Number of Independent plant	Seed hair length (mm)
WT	3	0.300±0.019
35S:expansin	5	0.357±0.017 (Figure 8b)

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It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

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Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications and GenBank Accession numbers mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application or GenBank Accession number was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be

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construed as an admission that such reference is available as prior art to the present invention.

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PCT/IL2005/000627

### WHAT IS CLAIMED IS:

WO 2005/121364

- 1. An isolated polynucleotide comprising a nucleic acid sequence encoding a polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96, wherein said polypeptide is capable of regulating cotton fiber development.
- 2. The isolated polynucleotide of claim 1, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NOs. 1, 2, 4, 5, 7, 9, 10, 16, 17, 20, 21, 22, 24, 25, 27 and 13.
- 3. The isolated polynucleotide of claim 1, wherein said polypeptide is as set forth in SEQ ID NO. 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96.
- 4. The isolated polynucleotide of claim 1, wherein said amino acid sequence is as set forth in SEQ ID NO. 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96.
- 5. The isolated polynucleotide of claim 1, wherein said cotton fiber development comprises fiber formation.
- 6. The isolated polynucleotide of claim 1, wherein said cotton fiber development comprises fiber elongation.
- 7. An isolated polynucleotide comprising a nucleic acid sequence at least 80 % identical to SEQ ID NO: 85 or 91, wherein said nucleic acid sequence is capable of regulating expression of at least one polynucleotide sequence operably linked thereto in an ovule endothelial cell.
- 8. The isolated polynucleotide of claim 7, wherein said ovule endothelial cell is of a plant fiber or a trichome.

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- 9. An oligonucleotide capable of specifically hybridizing to the isolated polynucleotide of claim 1 or 7.
- 10. A nucleic acid construct comprising the isolated polynucleotide of claim 1.
- 11. A nucleic acid construct comprising the isolated polynucleotide of claim 7.
- 12. The nucleic acid construct of claim 10, wherein the nucleic acid construct further comprising at least one cis-acting regulatory element operably linked to the isolated polynucleotide.
- 13. The nucleic acid construct of claim 7, wherein said polynucleotide sequence is selected from the group consisting of SEQ ID NOs: 1, 2, 4, 5, 7, 9, 10, 16, 17, 20, 21, 22, 24, 25, 27 and 13.
- 14. The nucleic acid construct of claim 12, wherein said cis-acting regulatory element is as set forth in SEQ ID NO: 74, 75, 85 or 91 or functional equivalents thereof.
- 15. A transgenic cell comprising the nucleic acid construct of claim 10 and/or 11.
- 16. A transgenic plant comprising the nucleic acid construct of claim 10 and/or 11.
- 17. A method of improving fiber quality and/or yield of a fiber producing plant, the method comprising regulating an expression level or activity of at least one polynucleotide encoding a polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96 in the fiber producing plant, thereby improving the quality and/or yield of the fiber producing plant.

- 18. The method of claim 17, wherein the quality of the fiber producing plant comprises at least one parameter selected from the group consisting of fiber length, fiber strength, fiber weight per unit length, maturity ratio, uniformity and micronaire.
- 19. The method of claim 17, wherein said regulating expression or activity of said at least one polynucleotide is up-regulating.
- 20. The method of claim 19, wherein said up-regulating is effected by introducing into the cotton the nucleic acid construct of claim 10 and/or 11.
- 21. The method of claim 17, wherein said regulating expression or activity of said at least one polynucleotide is down-regulating.
- 22. The method of claim 21, wherein said down-regulating is effected by gene silencing.
- 23. The method of claim 22, wherein said gene silencing is effected by introducing into the cotton the oligonucleotide of claim 9.
- 24. The method of claim 17, wherein said fiber producing plant is selected from the group consisting of cotton, silk cotton tree (Kapok, Ceiba pentandra), desert willow, creosote bush, winterfat, balsa, ramie, kenaf, hemp, roselle, jute, sisal abaca and flax.
- 25. A method of increasing a biomass of a plant, the method comprising regulating an expression level or activity of at least one polynucleotide encoding a polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96 in the plant, thereby increasing the biomass of the plant.
  - 26. The method of claim 25, wherein the plant is a monocot plant.

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- 27. The method of claim 25, wherein the plant is a dicot plant.
- 28. A method of identifying genes which are involved in cotton fiber development, the method comprising:
  - (a) providing expressed nucleic acid sequences derived from cotton fibers;
  - (b) providing expressed nucleic acid sequences derived from an ovule tissue;
  - (c) computationally assembling said expressed nucleic acid sequences of
    (a) and (b) to generate clusters; and
  - (d) identifying clusters of said clusters which comprise expressed nucleic acid sequences of (a) and (b), thereby identifying genes which are involved in cotton fiber development.
- 29. The method of claim 28, further comprising identifying genes which are differentially expressed in said cotton fiber following (d).
- 30. The method of claim 29, wherein said differentially expressed comprises:
  - (a) specific expression; and/or

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- (b) change in expression over fiber development.
- 31. A method of producing an insect resistant plant, comprising regulating an expression level or activity of at least one polynucleotide encoding a polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96 in a trichome of the plant, thereby producing the insect resistant plant.
  - 32. A method of producing cotton fibers, the method comprising:
  - (a) generating a transgenic cotton plant expressing at least one polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96; and

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(b) harvesting the fibers of said transgenic cotton plant, thereby producing the cotton fibers.

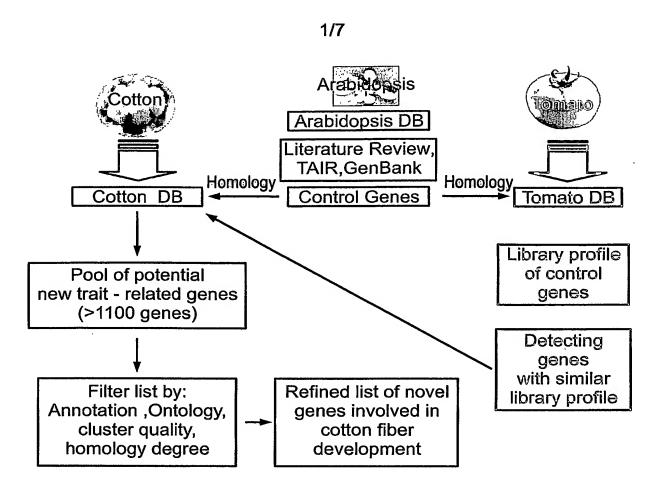
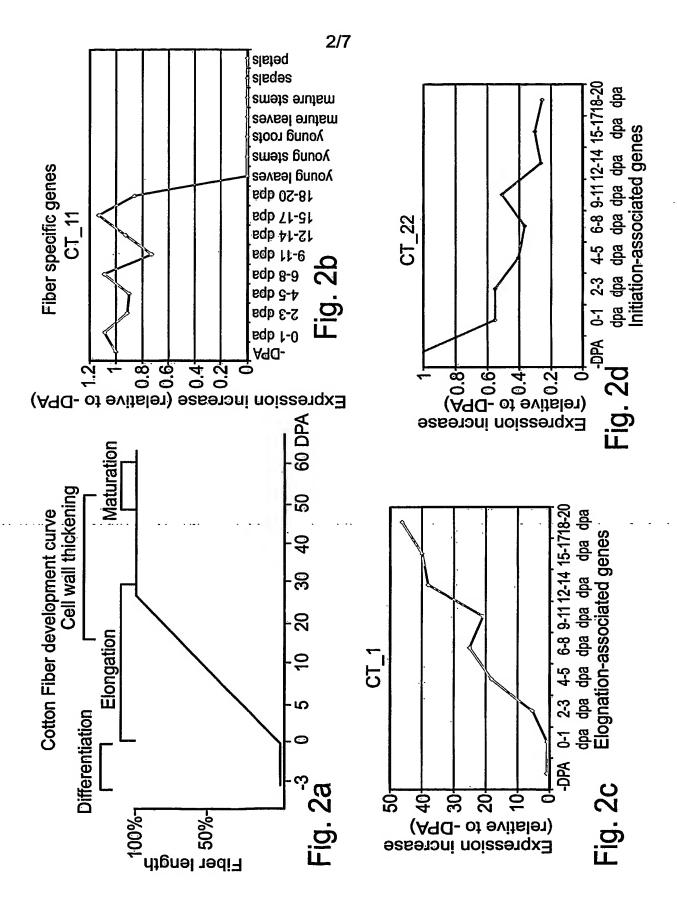


Fig. 1



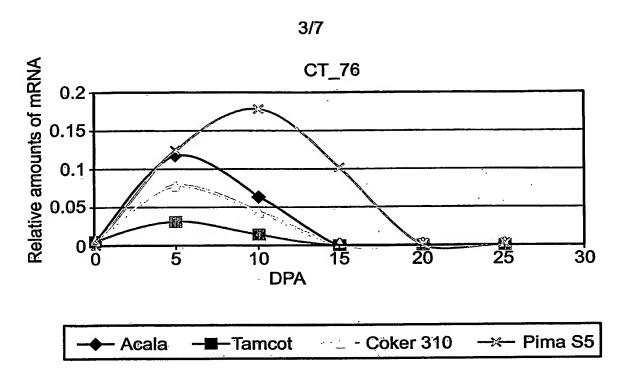
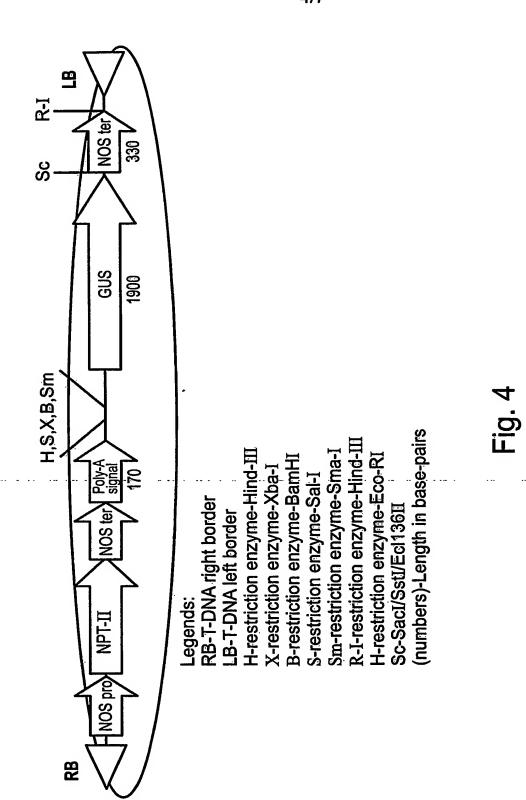
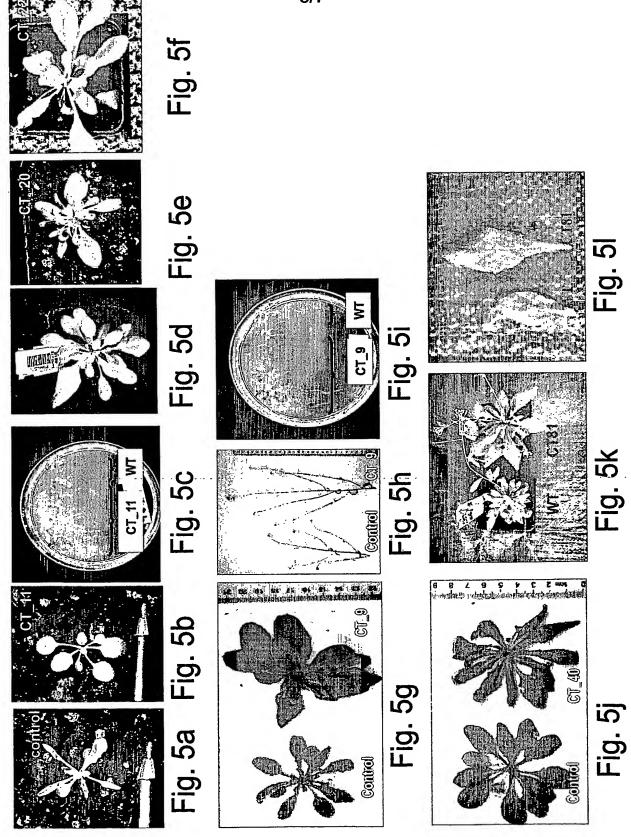
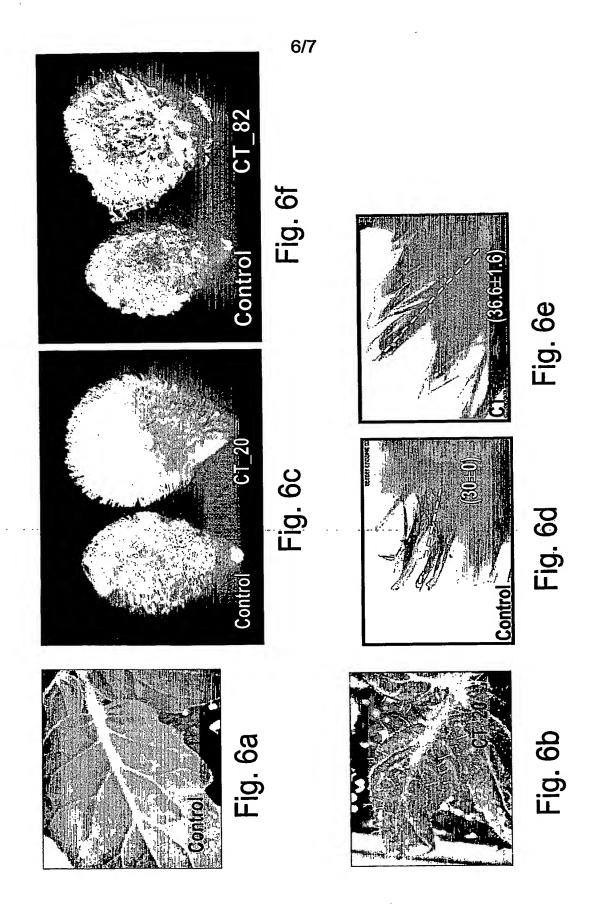


Fig. 3



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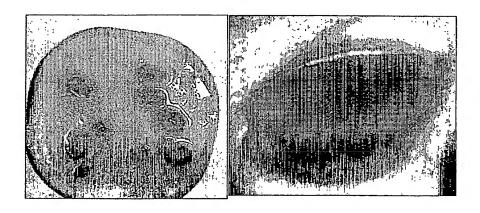


Fig. 7a

Fig. 7b

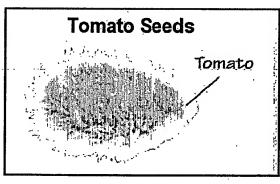


Fig. 8a

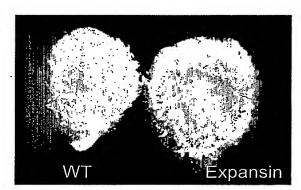


Fig. 8b

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ccttaaa	aatg taacacattt tetettgttt tggagtteat tttecaaatg gaaaatgttt	180
teegeea	aatc aaaagctgaa aaagttaaaa atgattttct ggaaaatcaa ttccgtcaat	240
caaaca	gacc cttagtctat tctctccaat taaatcattc ttagtcctta tacttttta	300
aatttci	tatc tcgatacaaa agacaaccat tgaatctatt aaattacctt tgtgtaaatg	360
atatat	gaaa ataataaatt gatatgacat aacgcatgcg ataatatatg taaaaatcac	420
caatta	cagg tacaaaaaaa tggttatgga ctaaatccgt aacttgcgca tgataaacga	480
agtggca	ataa tggataattc agtgttttac aatgtcaaaa tagcagcacc gtaatcgaac	540
atgatad	cett ggtecagttg tgctgtttac cgttggtata gtatttctac teteteteta	600
taaagag	gaga acgggacaaa catcatcccc accgctatgc ctattccccc actcaaattc	660
atttca	cttt taaataccaa ttaatattac ttacacttac ttccccttta caaatagata	720
atccaa	aagc-agagcaaaaa.cagagataac-cattctttt cttttgttgt tgttgttgtc	-780
gtcgtc	ggat cc	792
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<210> <211> <212>	94 1969 DNA	
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tttttgtcat	gaatcaatag	ttattgtagc	aatagttatc	tcttagccac	aataaattat	180
ttaaaataaa	atattatagc	taaataaata	tttttgcttt	aagttctaaa	agcttgtggc	240
aatagttaaa	tgatatagtc	acagatttat	tggtataatt	gaattatgtt	gctaatttct	300
tagtttttg	ccacgagtta	aaaattacca	atagctatag	taactttta	atcacaataa	360
aatatttgaa	agaaaatatt	gtagctaaat	gaatatttt	tccttcaagt	tattaaaagt	420
tgtggcaata	taggttaaat	tagccacatg	tttcttgctt	taatagaatt	ttgtagctaa	480
tcattaactt	ttaccacgag	ttgaacttaa	tataacaaca	ataacctttt	aaccataata	540
aagcgattta	aatcaaatat	tactaaataa	ataactttgc	tttcaagttt	ctataaaatc	600
atggcaatag	tcattacgat	aaaatgatat	aaccacgaat	atattgcaac	gataaattct	660
gtaactaatc	attagttttt	gcgacgaggt	aaattttccg	tcacagtagc	aatcttctag	720
gcacattaaa	aatttgaaac	aaaattttgt	agtcaaataa	atatttatct	tcttatttta	780
agaaaataaa	aatagttaga	taatagttac	tactatttgt	catgaaaata	tcaatagata	. 840
caaatttaaa	gtgactataa	atttacgagt	ttactatact	ttagtcgtac	agtttgcaat	900
aatagtattt	taaccacaat	tagttatatg	tacaaaataa	cataagtgaa	taacttttt	960
tcaatgagaa	aataagagtt	gctcaaacaa	tatcaagtta	caaaaattta	attttaactg	1020
taaaagttat	atttttccaa	aataacataa	actatagtaa	ttatatatag	tttgaagtat	1080
taataaaatt	taaatatgca	aaagttaatt	ttaataaacc	atttgtatgc	ctaacttgta	1140
gcctctaaac	tattttattt	gctttattta	tcaaactcat	attttattt	attgcacctt	1200
gttagttttg	gacgttaatt	atatatattt	ggtgtaaaat	ttaaaatata	ttaacatttg	1260
tggagaattt	atgtatgcct	.ggttcttaac	tatttttt	tatataactg	gttagagtaa	13 <u>2</u> 0
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cgctgttatt	taactgttta	tttcccttgt	taatataatt	ggtaagaagt	tttaaaataa	1500
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tttttttaga	gaaatcccat	ttcttgccta	aacgaaagco	: tataaaagag	catatattgc	1800
aacaacagtt	tgcagaaact	atcaagtcaa	ataatcccc	ctttaattcc	ctcccaaaat	1860
gcagttcttc	: aacttcttt	cccttttcct	ttttgtgtca	tttctctttt	tatttaagaa	1920
atggaagaat	tccaatagcc	aaaccaaaag	attgcctcca	ggtcccggg	-	1969

<sup>&</sup>lt;210> 95. <211> 198

Met Val Arg Gly Lys Ile Gln Met Lys Arg Ile Glu Asn Ala Thr Ser

<sup>&</sup>lt;212> PRT <213> Gossypium hirsutum

1 5 10 15

Arg Gln Val Thr Phe Ser Lys Arg Arg Asn Gly Leu Leu Lys Lys Ala 20 25 30

Tyr Glu Leu Tyr Val Leu Cys Asp Ala Glu Val Ala Val Ile Ile Phe 35 40 45

Ser His Lys Gly Lys Leu Tyr Glu Phe Ser Ser Ser Asp Asn Met Gln 50 60

Asn Thr Ile Glu Arg Tyr Arg Gln Tyr Lys Lys Asp Val Gln Ser Asn 65 70 75 80

Ile Pro Glu Phe Asp Arg Tyr Thr Gln Gln Leu Arg Leu Glu Ala Glu 85 90 95

Asn Met Ala Lys Lys Ile Glu Phe Leu Glu Val Ser Lys Arg Arg Met 100 105 110

Leu Gly Gln Asn Leu Gly Ser Cys Ser Ile Asp Glu Leu Gln Glu Val 115 120 125

Glu Asn Gln Leu Glu Arg Ser Leu Arg Asn Ile Arg Ala Arg Lys Gly 130 135 140

Tyr Leu Phe Lys Glu Gln Ile Leu Gln Leu Lys Ala Lys Glu Arg Tyr 145 150 155 160

Met Gln Glu Glu Asn Ala Lys Leu Ser Ala Lys Asn Asn Gly Thr Thr 165 170 175

Cys Ser Gln Gln Asn Ala Glu Val Glu Thr Glu Leu Phe Leu Gly Leu

Pro Glu Asn Arg Cys Ser 195

<210> 96

<211> 463

<212> PRT

<213> Gossypium hirsutum

<400> 96

Met Lys Arg Val Ser Asn Ile Tyr Ile Glu Ala Lys Lys His Leu Ser 1 10 15

Asp Leu Leu Thr Asn Glu Asp Gln Asn Glu Asp Leu Leu Ser Thr Gln 20 25 30

Val Pro Lys Thr Leu Gly Arg Ile Leu Ser Leu Pro Glu Tyr Asn Thr 35 40 45

Ser Pro Val Ser Ser Pro Gly Gln Asn Leu Glu His Ser Phe Thr Thr 50 55 60

Ala His Met Arg Phe Ala Gly Ser Asp Lys Leu Gln Met Val Ser Glu 65 70 75 80

- Asn Asp Arg Phe Val Ser Leu Leu Ser Met Arg Ala Glu Lys Thr Asp 85 90 95
- Gly Gln Leu Cys Ile Ser Glu Asn Lys Ser Asp Asn Glu Val Glu Ser 100 105 110
- Asp Asn Ala Ile Ser Asn Asn Leu Asp Thr Ser Val Asn Asn Asp Lys 115 120 125
- Glu Asp Pro Ile Phe Cys Ser Ile Lys Asp Glu Leu Ser Ser Lys Glu 130 135 140
- Ser Val Ser Ile Val Lys Ala Thr Glu Met Met Val His Glu Glu Ser 145 150 155 160
- Lys Ser Leu Asp Ile Ser Ser Glu Thr Ser Gly Ser Ser Ile Ile Thr 165 170 175
- Asp Asp Lys Asn Val Asp Ile Tyr Glu Val Cys Asp Glu Lys Gln Asn 180 185 190
- Pro Trp Tyr Leu Lys Gln Asp Ser Ser Glu Val Asp Gln Gln Pro Phe 195 200 205
- Ser Pro Leu Ser Ser Pro Ser Asp Ser Ser Val Met Lys Lys Val Glu 210 . 215 220
- Cys Leu Glu Ser Val Thr Asp Ile Pro Glu Arg Ser Ser Pro Val Ser 225 230 235 240
- Val Leu Glu Pro Ile Phe Ala Asp Asp Leu Ile Ser Pro Ala Ser Ile 245 250 255
- Arg Ser Tyr Ser Gly Glu Thr Ser Ile Gln Pro Leu Arg Ile Arg Phe 260 265 270
- Glu Glu His Asp Ser Leu Ala Thr Asn Gln Ser Asn Arg Ile Lys Thr 275 280 285
- Cys Met Asn Asp Lys Glu Ser Ile Phe Glu His Ile Lys Ala Val Leu 290 295 300
- Gln Ala Ser Ser Phe Ser Trp Asp Glu Val Tyr Ile Arg Ser Leu Ser 305 310 315 320
- Ser Asp Leu Leu Ile Asp Pro Leu Leu Val Asp Glu Val Glu Tyr Leu 325 330 335
- Pro Asn Gln Leu Cys Gln Asp Gln Lys Leu Phe Asp Cys Ile Asn 340 345 350
- Glu Val Val Arg Glu Val Cys Glu Tyr Tyr Phe Gly Ser Pro Ser Val 355 360 365
- Ser Phe Val Lys Pro Asn Ile Arg Pro Ile Pro Asn Met Gln Asn Thr · 370 375 380

Ile Gln Glu Val Trp Glu Gly Val Tyr Trp His Leu Leu Pro Thr Pro 390 395 385 Leu Pro Cys Thr Leu Asp Leu Val Val Arg Lys Asp Leu Ala Lys Thr 410 Gly Thr Trp Met Asp Leu Gln Leu Asp Thr Gly Tyr Ile Gly Val Glu Ile Gly Glu Ala Ile Phe Glu Asp Leu Val Glu Asp Thr Ile Thr Ser Tyr Ile Asn Gly Ser Trp Glu Cys Glu Tyr Asn Val Leu Pro Ala <210> 97 <211> 21 <212> DNA <213> Artificial sequence <220> <223> Single strand DNA oligonucleotide 21 tcagattccg ccttgatagc a <210> 98 <211> 23 <212> DNA <213> Artificial sequence <220> <223> Single strand DNA oligonucleotide · <400> 98 ctctgtggtt gacctcactg tga 23 ..... ··· <210>··99 · <211> 23 <212> DNA <213> Artificial sequence <223> Single strand DNA oligonucleotide <400> 99 23 cctaccaaga ttcactcccg atc <210> 100 <211> 24 <212> DNA <213> Artificial sequence <220> <223> Single strand DNA oligonucleotide <400> 100 · cctcttacca tagccagaat gacg 24 <210> 101 <211> 21 <212> DNA <213> Artificial sequence <220> <223> Single strand DNA oligonucleotide

<400> 101

tccacaa	acat tgacgtcage c	21
-010>	102	
<210> <211>	102 21	
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ctaatag	gaag cccagcagtg g	21
40105	103	
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	DNA	
	Artificial sequence	
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	Single strand DNA oligonucleotide	
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<210> <211>	104 23	
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	Artificial sequence	
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	Single strand DNA oligonucleotide	
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tttccc	ggga cctggaggca atc	23
	•	
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<211>		
<212> <213>		
- < 4.0.0>	105	
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tttttg	tcat gaatcaatag ttattgtagc aatagttatc tcttagccac aataaattat	180 ·
	taaa atattatagc taaataaata tttttgcttt aagttctaaa agcttgtggc	240
_		300
	taaa tgatatagtc acagatttat tggtataatt gaattatgtt gctaatttct	
tagttt	tttg ccacgagtta aaaattacca atagctatag taacttttta atcacaataa	360
aatatt	tgaa agaaaatatt gtagctaaat gaatattttt tccttcaagt tattaaaagt	420
tgtggc	aata taggttaaat tagccacatg tttcttgctt taatagaatt ttgtagctaa	480
tcatta	actt ttaccacgag ttgaacttaa tataacaaca ataacctttt aaccataata	540
aagcga	ttta aatcaaatat tactaaataa ataactttgc tttcaagttt ctataaaatc	600
	atag tcattacgat aaaatgatat aaccacgaat atattgcaac gataaattct	660
	aatc attagttttt gcgacgaggt aaattttccg tcacagtagc aatcttctag	720
gcacat	taaa aatttgaaac aaaattttgt agtcaaataa atatttatct tcttatttta	780
agaaaa	taaa aatagttaga taatagttac tactatttgt catgaaaata tcaatagata	840
caaatt	taaa gtgactataa atttacgagt ttactatact ttagtcgtac agtttgcaat	900
aatagt	attt taaccacaat tagttatatg tacaaaataa cataagtgaa taacttttt	960

tcaatgagaa	aataagagtt	gctcaaacaa	tatcaagtta	caaaaattta	attttaactg	1020
taaaagttat	atttttccaa	aataacataa	actatagtaa	ttatatatag	tttgaagtat	1080
taataaaatt	taaatatgca	aaagttaatt	ttaataaacc	atttgtatgc	ctaacttgta	1140
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gttagttttg	gacgttaatt	atatatattt	ggtgtaaaat	ttaaaatata	ttaacatttg	1260
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tttttttaga	gaaatcccat	ttcttgccta	aacgaaagcc	tataaaagag	catatattgc	1800
aacaacagtt	tgcagaaact	atcaagtcaa	ataatcccc	ctttaattcc	ctcccaaaat	1860
gcagttcttc	aacttcttt	cccttttcct	ttttgtgtca	tttctctttt	tatttaagaa	1920
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<sup>&</sup>lt;210> 106

<400> 106

Met Asp Gly Tyr Cys Ser Arg Arg Val Ile Met Phe Leu Val Phe Ala 5 10 15

Phe Ala Ala Ile Ser Arg Gly Tyr Gly Gln Glu Ser Thr Thr Leu Val

Pro Ala Ile Ile Thr Phe Gly Asp Ser Val Val Asp Val Gly Asn Asn

Asp Tyr Leu Pro Thr Ile Phe Lys Ala Asn Tyr Pro Pro Tyr Gly Arg

· Asp Phe Ala Asn Lys Lys Pro Thr Gly Arg Phe Cys Asn Gly Lys Leu

Ala Thr Asp Ile Thr Ala Glu Thr Leu Gly Phe Thr Thr Tyr Pro Pro

Ala Tyr Leu Ser Pro Glu Ala Ser Gly Lys Asn Leu Leu Gly Ala

Asn Phe Ala Ser Ala Gly Ser Gly Tyr Asp Asp Lys Ala Ala Met Val

. Asn His Ala Ile Thr Leu Thr Gln Gln Leu Glu Tyr Phe Lys Glu Tyr

<sup>&</sup>lt;211> 356 <212> PRT

<sup>&</sup>lt;213> Gossypium hirsutum

Gin Ala Lys Leu Ala Lys Val Ala Gly Ser Thr Lys Ser Ala Ser Ile 150

Thr Lys Asp Ala Leu Tyr Val Leu Ser Ala Gly Ser Gly Asp Phe Leu

Gln Asn Tyr Tyr Val Asn Pro Leu Leu Asn His Ala Tyr Thr Pro Asp 185

Gln Tyr Gly Ser Phe Leu Ile Asp Thr Phe Thr Asn Phe Val Lys Asn

Leu Tyr Gly Leu Gly Ala Arg Lys Ile Gly Val Thr Ser Leu Pro Pro

Leu Gly Cys Val Pro Leu Ala Arg Thr Leu Phe Gly Tyr His Glu Lys

Gly Cys Ile Ser Arg Phe Asn Thr Asp Ala Gln Gln Phe Asn Lys Lys

Leu Asn Ala Ala Ala Asn Leu Gln Lys Gln His Pro Gly Leu Lys

Ile Val Val Phe Asp Ile Phe Lys Ala Leu Tyr Asp Ile Val Lys Ser

Pro Ser Asn Tyr Gly Phe Val Glu Ala Thr Lys Gly Cys Cys Gly Thr

Gly Thr Val Glu Thr Thr Ala Phe Leu Cys Asn Pro Lys Ala Pro Gly 315 310

. ... . Thr Cys Ser Asn Ala Ser Gln Tyr Val Phe Trp Asp Ser Val His Pro

Ser Gln Ala Ala Asn Gln Val Leu Ala Asp Ala Leu Ile Val Gln Gly 345

Ile Ala Leu Ile 355

.....

<210> 107

<211> 645

<212> PRT

<213> Gossypium hirsutum

Met Glu Ala Ser Ser Ser Thr Ser His Asp Pro Ala Leu Phe His Ala

Pro Leu Leu Tyr His Pro Arg Arg Ser Ser Arg Pro Leu Lys Gly

Phe Ala Val Ile Ile Gly Ser Val Val Phe Leu Leu Ser Leu Val Thr

Leu Ile Val Asn Gln Ser Pro Glu Pro Leu Ala Ser Asn Pro Ser Ser

WO 2005/121364

60

/al	Thr	Glu	Ala	Gly	Ser 70	Tyr	Ser	Met	Ala	Ala 75	Gln	Pro	Arg	Gly	Ile 80
99					, ,										

55

50

Ala Glu Gly Val Ser Ala Lys Ser Asn Pro Ser Leu Phe Asp Lys Val 85 90 95

Gly Phe Asn Trp Thr Asn Ala Met Phe Tyr Trp Gln Arg Thr Ala Tyr 100 105 110

His Phe Gln Pro Gln Lys Asn Trp Met Asn Asp Pro Asp Gly Pro Leu 115 120 125

Tyr His Lys Gly Trp Tyr His Leu Phe Tyr Gln Tyr Asn Pro Asp Ser

Ala Ile Trp Gly Asn Ile Thr Trp Gly His Ala Val Ser Thr Asp Leu 145 150 155 160

Ile His Trp Phe Tyr Leu Pro Leu Ala Met Val Pro Asp Gln Trp Tyr 165 170 175

Asp Ile Asn Gly Cys Trp Thr Gly Ser Ala Thr Leu Leu Pro Asp Gly
180 185 190

Arg Ile Val Met Leu Tyr Thr Gly Ser Thr Asn Asp Ser Val Gln Val 195 200 205

Gln Asn Leu Ala Tyr Pro Ala Asn Leu Ser Asp Pro Leu Leu Gln 210 215 220

Glu Asp Glu Glu Phe Arg Asp Pro Thr Thr Ala Trp Leu Gly Pro Asp 245 250 255

Gly Ser Trp Arg Ile Val Val Gly Thr Arg Phe Asn Thr Thr Ile Gly 260 265 270

Thr Ala Leu Val Phe Gln Thr Thr Asn Phe Ser Asp Tyr Glu Leu Leu 275 280 285

Asp Gly Val Leu His Ala Val Pro Gly Thr Gly Met Trp Glu Cys Val 290 295 300

Asp Phe Tyr Pro Val Ala Ile Asn Gly Ser Val Gly Leu Asp Thr Thr 305 310 315 320

Ala Leu Gly Pro Gly Ile Lys His Val Leu Lys Ala Ser Leu Asp Asp 325 330 335

Thr Lys Val Asp His Tyr Ala Ile Gly Thr Tyr Asp Met Ile Thr Asp 340 345 350

Lys Trp Thr Pro Asp Asn Pro Glu Glu Asp Val Gly Ile Gly Leu Lys 355 360 365

Val Asp Tyr Gly Arg Tyr Tyr Ala Ser Lys Thr Phe Phe Asp Gln Ser 370 375 380

Lys Gln Arg Arg Ile Leu Tyr Gly Trp Val Asn Glu Thr Asp Ser Glu 385 390 395 400

Ala Asp Asp Leu Glu Lys Gly Trp Ala Ser Ile Gln Thr Ile Pro Arg 405 410 415

Ser Val Leu Tyr Asp Asn Lys Thr Gly Thr His Leu Leu Gln Trp Pro 420 425 430

Val Glu Glu Val Glu Ser Leu Arg Leu Asn Ala Thr Val Phe Lys Asp 435 440 445

Val Val Glu Ala Gly Ser Val Val Pro Leu Asp Ile Gly Thr Ala 450 455 460

Thr Gln Leu Asp Ile Leu Ala Glu Phe Glu Ile Glu Thr Leu Val Leu 465 470 475 480

Asn Ser Thr Glu Asp Glu Val Ser Asp Cys Gly Asp Gly Ala Val Asp 485 490 495

Arg Ser Thr Tyr Gly Pro Phe Gly Val Leu Val Ile Ala Asp Asp Ser 500 505 510

Leu Ser Glu Leu Thr Pro Ile Tyr Phe Arg Pro Leu Asn Thr Ser Asp 515 520 525

Gly Ser Leu Glu Thr Tyr Phe Cys Ala Asp Glu Thr Arg Ser Ser Lys
530
540

Ala Pro Asp Val Thr Lys Arg Val Tyr Gly Gly Lys Ile Pro Val Leu 545 550 560

Asp Asp Glu Asn Tyr Asn Met Arg Val Leu Val Asp His Ser Val Val 565 570 575

Glu Ser Phe Gly Gly Gly Gly Arg Thr Val Ile Thr Ser Arg Val Tyr
580 585 590

Pro Thr Glu Ala Ile Tyr Gly Ala Ala Arg Leu Phe Leu Phe Asn Asn 595 600 605

Ala Ser Gly Val Asn Val Lys Ala Thr Leu Lys Ile Trp Glu Met Asn 610 . 620

Ser Ala Phe Ile Arg Pro Phe Pro Phe Glu Glu Thr Leu Phe Gln Glu 625 630 635 640

Met Val Ala Ser Thr 645

<210> 108 <211> 180 <212> PRT <213> Gossypium hirsutum

<400> 108

Met Glu Leu Ser Ile Gln Lys Ile Glu Ala Leu Ile Arg Leu Ser Thr 1 5 10 15

Ile Val Met Leu Val Leu Thr Ala Cys Leu Ile Gly Leu Asp Ser Gln 20 25 30

Thr Lys Val Ile Phe Tyr Val Gln Lys Lys Ala Ser Phe Lys Asp Leu 35 40 45

Arg Ala Leu Val Gly Leu Leu Tyr Ile Thr Ser Leu Ala Ala Ala Tyr 50 60

Asn Leu Leu Gln Leu Cys Cys Ser Ser Phe Ser Ala Ser Tyr Lys Gly 65 70 75 80

Thr Ser Leu Gln Ser Tyr Ala Tyr Leu Ala Trp Leu Arg Tyr Ile Leu 85 90 95

Asp Gln Ala Val Val Tyr Ala Val Phe Ala Gly Asn Leu Ala Ala Leu 100 105 110

Glu His Ser Phe Leu Val Leu Thr Gly Glu Glu Asn Phe Gln Trp Leu 115 120 125

Lys Trp Cys Asn Lys Tyr Thr Arg Phe Cys Thr Gln Ile Gly Gly Ser 130 135 140

Leu Leu Cys Gly Phe Val Ala Ser Leu Leu Met Phe Ser Ile Ala Ser 145 . 150 . 155 . 160

Ile Ser Ala Phe Asn Leu Phe Arg Leu Tyr Ser Pro Thr Lys Phe Met .. ... 165 170 175

His Leu Lys Leu 180

<210> 109

<211> 189 <212> PRT

<212> FKI <213> Gossypium hirsutum

<400> 109

Met Ala Glu Ile Leu Arg Lys Pro Ser Val Leu Lys Lys Leu Leu Leu 1 5 10 15

Glu Leu Asp Gln Val Val Gly Lys Asp Arg Phe Val Val Glu Ser Asp

Ile Pro Lys Leu Thr Tyr Leu Gln Ala Val Val Lys Glu Val Phe Arg

Leu His Pro Gly Val Pro Leu Ile Ile Pro Arg Arg Thr Asn Glu Ala 50 55 60

Cys Glu Val Ala Gly Tyr His Ile Pro Lys His Cys Ile Val Tyr Val 65 70 75 80

Asn Val Trp Gly Met Ala Arg Asp Pro Asn Val Trp Glu Asp Pro Leu 85 90 95

Glu Phe Lys Pro Glu Arg Phe Ile Gly Ser Ser Val Asp Val Lys Gly  $100 \hspace{1cm} 105 \hspace{1cm} 110$ 

Gln Asp Phe Asn Leu Leu Pro Phe Gly Thr Gly Arg Arg Ser Cys Val 115 120 ` 125

Gly Trp Pro Leu Ala His Arg Met Val His Tyr Tyr Leu Ala Ala Leu 130 135 140

Leu His Ala Phe Gln Trp Glu Ser Pro Pro Asp Val Leu Asn Asp Leu 145 150 155 160

Gly Glu Arg Val Gly Leu Thr Ile Gln Lys Gly Lys Ser Leu Leu Ser 165 170 175

Thr Pro Lys Pro Arg Leu Pro Ala Ser Val Tyr Glu Arg 180 185

<210> 110

<211> 468

<212> PRT

<213> Gossypium hirsutum

<400> 110

Met Ala Ser Leu Pro Phe Ile Phe Phe Leu Ser Phe Phe Ile Ile Ser 1 5 10 15

Thr Thr Leu Thr Ser Ala Gly Ala Ala Ala Ala Thr Ile Lys Leu Ser 20 25 30

Leu Ser Pro Phe Pro His Pro Ser Ser Ser His Pro Tyr Gln Ile Leu 35 40 45

Asn Asn Leu Val Thr Ser Ser Val Ala Arg Ala His His Leu Lys His 50 60

Pro Lys Ala Lys Ala Asp Asn Thr Thr Ser Ser Leu Leu Arg Ala Pro 65 70 75 80

Leu Phe Ser His Ser Tyr Gly Gly Tyr Thr Ile Ser Leu Lys Phe Gly 85 90 95

Thr Pro Pro Gln Thr Leu Pro Phe Val Met Asp Thr Gly Ser Ser Leu
100 105 110

Ser Trp Phe Pro Cys Thr Ser Arg Tyr Leu Cys Ser Gln Cys Ala Phe 115 120 125

Pro Asn Val Asp Pro Ala Lys Ile Pro Thr Phe Ala Pro Lys Leu Ser 130 135 140

Ser Ser Ser Lys Leu Val Gly Cys Arg Asn Pro Lys Cys Ser Trp Leu 145 150 155 160

- Phe Gly Pro Asp Val Glu Ser Arg Cys Gln Asp Cys Glu Pro Thr Ser 165 170 175
- Glu Asn Cys Thr Gln Thr Cys Pro Pro Tyr Ile Ile Gln Tyr Gly Leu 180 185 190
- Gly Ser Thr Ala Gly Leu Leu Leu Val Glu Asn Leu Ala Phe Pro Gln 195 200 205
- Lys Thr Phe Gln Asp Phe Leu Val Gly Cys Ser Ile Leu Ser Asn Arg 210 215 220
- Gln Pro Ala Gly Ile Ala Gly Phe Gly Arg Ser Ala Glu Ser Ile Pro 225 230 235 240
- Ser Gln Leu Gly Leu Lys Lys Phe Ser Tyr Cys Leu Val Ser Arg Arg 245 250 255
- Phe Asp Asp Thr Gly Val Ser Ser Asn Met Leu Leu Glu Thr Gly Ser 260 265 270
- Gly Ser Gly Asp Ala Lys Thr Pro Gly Leu Ser Tyr Thr Pro Phe Tyr 275 280 285
- Arg Asn Gln Val Ala Ser Asn Pro Val Phe Lys Glu Phe Tyr Tyr Val 290 295 300
- Thr Leu Arg Lys Ile Leu Val Gly Asp Lys His Val Lys Val Pro Tyr 305 310 315 320
- Ser Tyr Leu Val Pro Gly Ser Asp Gly Asn Gly Gly Thr Ile Val Asp 325 330 335
- ----Ser Gly-Ser Thr Phe Thr Phe-Met Glu Arg Pro Val Phe Glu Val -- 340 345 350
  - Ser Lys Glu Phe Glu Lys Gln Met Gly Asn Tyr Arg Arg Val Arg Glu 355 360 365
  - Ile Glu Asn Arg Ser Gly Leu Ala Pro Cys Phe Asn Thr Ser Gly Tyr 370 375 380
  - Thr Ser Ile Glu Ile Pro Glu Leu Ser Phe Gln Phe Lys Gly Gly Ala 385 390 395 400
  - Lys Met Ala Leu Pro Leu Val Asn Tyr Phe Ser Phe Asp Gly Asp Asp 405 410 415
  - Lys Val Val Cys Leu Met Ile Val Ser Asn Asn Val Val Gly Gln Gly 420 425 430
  - Ser His Ser Gly Pro Ala Ile Ile Leu Gly Ser Phe Gln Gln Asn 435 440 445
  - Tyr Tyr Ile Glu Phe Asp Ile Ala Asn Asn Arg Phe Gly Trp Ala Glu 450 460

Arg Ser Cys Ala

465

<210> 111

<211> 451 <212> PRT

<213> Gossypium hirsutum

<400> 111

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Ile Thr Gly Lys Ser His Val Lys Pro Gly Lys Leu Ile Gly Arg Lys
20 25 30

Glu Cys Gln Leu Val Thr Phe Asp Leu Pro Tyr Leu Ala Phe Tyr Tyr 35 40 45

As Gln Lys Leu Leu Phe Tyr Lys As Asp Gly Gly Glu Phe Glu 50  $\,$  55  $\,$  60

Asp Lys Val Glu Lys Leu Lys Gly Gly Leu Arg Val Val Leu Glu Glu 65 70 75 80

Phe Tyr Gln Leu Gly Gly Lys Leu Gly Lys Asp Asp Gly Val Leu 85 90 95

Arg Val Asp Tyr Asp Asp Asp Met Asp Gly Val Glu Val Val Glu Ala 100 105 110

Val Ala Glu Gly Ile Thr Val Asp Glu Leu Thr Gly Asp Asp Gly Thr 115 120 125

Ser Ser Phe Lys Glu Leu Ile Pro Phe Asn Gly Val Leu Asn Leu Glu 130 135 140

Gly Leu His Arg Pro Leu Leu Ser Ile Gln Leu Thr Lys Leu Lys Asp 145 150 150 155 160

Gly Val Ala Met Gly Cys Ala Phe Asn His Ala Ile Leu Asp Gly Thr 165 170 175

Ser Thr Trp His Phe Met Ser Ser Trp Ala Gln Ile Cys Asn Gly Thr 180 185 190

Ser Ser Ser Val Val Val Pro Pro Phe Leu Asp Arg Thr Thr Ala Arg 195 200 205

Asn Thr Arg Val Lys Leu Asp Leu Ser Pro Val Val Ser Cys Asn Gly 210 215 220

Asp Asp Ala Thr Lys Gln Gly Gln Pro Ala Pro Gln Met Arg Glu Lys 225 230 235 240

Leu Phe Arg Phe Ser Glu Ala Ala Val Asp Lys Ile Lys Ser Arg Val .. 245 250 250

Asn Ser Thr Pro Pro Pro Ser Asp Gly Ser Lys Pro Phe Ser Thr Phe 260 265 270

Gln Ser Leu Ala Val His Ile Trp Arg His Val Ser Gln Ala Arg Asn 275 280 285

Leu Lys Pro Glu Asp Tyr Thr Val Phe Thr Val Phe Ala Asp Cys Arg 290 295 300

Lys Arg Val Asp Pro Pro Met Pro Asp Ser Tyr Phe Gly Asn Leu Ile 305 310 315 320

Gln Ala Ile Phe Thr Ala Thr Ala Ala Gly Leu Leu Leu Glu Asn Pro 325 330 335

Pro Ser Phe Gly Ala Ser Val Ile Gln Lys Ala Ile Glu Ser His Asp 340 345 350

Ala Lys Ala Ile Asp Glu Arg Asn Lys Ala Trp Glu Ala Ala Pro Lys 355 360 365

Ile Phe Gln Phe Lys Asp Ala Gly Val Asn Cys Val Ala Val Gly Ser 370 375 380

Ser Pro Arg Phe Lys Val Tyr Glu Val Asp Phe Gly Trp Gly Lys Pro 385 390 395 400

Val Gly Val Arg Ser Gly Ser Asn Asn Arg Phe Asp Gly Met Val Tyr 405 410 415

Leu Tyr Gln Gly Lys Ser Gly Gly Arg Ser Ile Asp Val Glu Ile Thr 420 425 430

Met Glu Ala Gln Ala Met Glu Lys Leu Glu Lys Asp Lys Glu Phe Leu
435 440 445

Met Glu Val 450

<210> 112

<211> 467 <212> PRT

<213> Gossypium hirsutum

<400> 112

Met Ser Thr Gln Ser Arg Ala Val Gly Gly Thr Glu His Asn Trp Cys

Arg Ala Val Val Gly Gly Thr Gly Ile Ala Val Leu Ala Ile Ile Ser 20 25 30

Ser Lys Asn Pro Asp Val Ser His Leu Lys Asn Ala Leu His Lys Leu 35 . 40 45

Gln Ile Ser His Pro Ile Leu Arg Ser Arg Leu His Tyr Ser Pro Thr 50 55 60

Ala Asn Ser Tyr Ser Phe Val Thr Ser Pro Ser Pro Phe Ile Gln Ile 65 70 75 80

Lys Tyr Phe Asn His Ser Thr Thr Cys Gln Ile Leu Glu Asn Asn Gln

85 90 95

Asn Ile Ser Pro Leu His Leu Ile Leu Glu His Glu Leu Asn Gln Asn 100 105 110

Ala Trp Val Ser Ser Ser Cys Thr Thr Lys His Asp Val Phe Phe Ala 115 120 125

Ser Val Tyr Ala Leu Pro Gly Ala Thr Arg Trp Val Leu Val Leu Arg 130 135 140

Leu His Ala Ala Ala Cys Asp Arg Thr Thr Ala Val Ser Leu Leu Arg 145 150 155 160

Glu Leu Leu Thr Leu Met Ala Ile Glu Glu Glu Glu Thr Gly Phe Gln 165 170 175

Gln Gly Gln Lys Glu Ile Thr Met Asn Lys Gly Glu Ile Ser Leu Ala 180 185 190

Met Glu Asp Ile Leu Pro Lys Gly Ile Val Lys Lys Thr Leu Trp Ala 195 200 205

Arg Gly Val Asp Met Leu Ser Tyr Ser Val Asp Ser Leu Arg Phe Thr 210 215 220

Asn Leu Arg Phe Lys Asp Ala Lys Ser Pro Arg Ser Thr Gln Val Val 225 230 235

Arg Leu Leu Ile Asn Pro Asp Asp Thr Gln Lys Ile Leu Thr Gly Cys 245 250 255

Lys Ala Arg Gly Ile Lys Leu Cys Gly Ala Leu Gly Ala Ala Gly Leu

Ile Ser Ala His Ser Ser Lys Ser Arg Ser Asp His Gln Lys Lys Lys 275 280 285

Tyr Gly Val Val Thr Leu Thr Asp Cys Arg Ser Ile Leu Glu Pro Pro 290 295 300

Leu Ser Asn His His Phe Gly Phe Tyr His Ser Ala Ile Leu Asn Thr 305 310 315 320

His Ala Ile Lys Gly Glu Lys Leu Trp Glu Leu Ala Glu Lys Val 325 330 335

Tyr Thr Val Phe Thr His Tyr Lys Ser Cys Asn Lys His Leu Ser Asp 340 · 345 350

Met Ala Asp Leu Asn Phe Leu Met Cys Arg Ala Met Glu Asn Pro Gly 355 360 365 .

Leu Thr Pro Ser Ala Ser Leu Arg Thr Cys Leu Ile Ser Val Phe Glu 370 375 380

Asp Thr Val Ile Asp Glu Ser Ser Asn Gln Gln Asn Gln Val Gly Val 385 390 395 400

PCT/IL2005/000627 WO 2005/121364

Glu Asp Tyr Met Gly Cys Ala Ser Ala His Gly Ile Ala Pro Ser Ile

Ala Ile Phe Asp Thr Ile Arg Asp Gly Arg Leu Asp Cys Ile Cys Val

Tyr Pro Ser Pro Leu His Ser Arg Glu Gln Met Gln Glu Leu Val Asp

Asn Met Lys Cys Ile Leu Val Asp Ala Gly Lys Asn Val Ala Asp Glu

Thr Glu Ser

<210> 113

<211> 223

<212> PRT <213> Gossypium hirsutum

Met Gly Arg Gly Lys Ile Glu Ile Lys Arg Ile Glu Asn Thr Thr Asn

Arg Gln Val Thr Phe Cys Lys Arg Arg Asn Gly Leu Leu Lys Lys Ala

Tyr Glu Leu Ser Val Leu Cys Asp Ala Glu Val Ala Leu Ile Val Phe

Ser Ser Arg Gly Arg Leu Tyr Glu Tyr Ser Asn Asn Asn Ile Arg Ser

Thr Ile Asp Arg Tyr Lys Lys Ala Cys Ser Asp Thr Ser Asn Thr Asn

Thr Val Thr Glu Ile Asn Ala Gln Tyr Tyr Gln Gln Glu Ser Ala Lys

Leu Arg Gln Gln Ile Gln Met Leu Gln Asn Ser Asn Arg His Leu Met

Gly Asp Ser Leu Ser Ser Leu Thr Val Lys Glu Leu Lys Gln Val Glu

Asn Arg Leu Glu Arg Gly Ile Thr Arg Ile Arg Ser Lys Lys His Glu <sub>.</sub> 135

Met Leu Leu Ala Glu Ile Glu Phe Leu Gln Lys Arg Glu Ile Glu Leu

Glu Asn Glu Ser Val Cys Leu Arg Thr Lys Ile Ala Glu Ile Glu Arg

Leu Gln Gln Ala Asn Met Val Thr Gly Pro Glu Leu Asn Ala Ile Gln 185

Ala Leu Ala Ser Arg Asn Phe Phe Ser Pro Asn Val Ile Glu His Pro 195 200 205

Ser Ala Tyr Ser His Leu Ser Asp Lys Lys Ile Leu His Leu Gly 210 215 220

<210> 114

<211> 310

<212> PRT

<213> Gossypium hirsutum

<400> 114

Met Asp Val Thr Ser Thr Pro Asn Arg Lys Glu Met Asp Arg Ile Lys 1 5 10 15

Gly Pro Trp Ser Pro Glu Glu Asp Asp Leu Leu Gln Gln Leu Val Gln 20 25 30

Lys His Gly Pro Arg Asn Trp Ser Leu Ile Ser Lys Ser Ile Pro Gly 35 40 45

Arg Ser Gly Lys Ser Cys Arg Leu Arg Trp Cys Asn Gln Leu Ser Pro 50 55 60

Gln Val Glu His Arg Ala Phe Thr Pro Glu Glu Asp Glu Thr Ile Ile 65 70 75 80

Arg Ala His Ala Arg Phe Gly Asn Lys Trp Ala Thr Ile Ala Arg Leu 85 90 95

Leu Asn Gly Arg Thr Asp Asn Ala Ile Lys Asn His Trp Asn Ser Thr 100 105 110

Leu Lys Arg Lys Cys Leu Pro Val Gly Glu Glu Cys Asn Phe Val Ala

Asn Gly Gly Tyr Asp Gly Asn Leu Gly Gly Glu Glu Arg Gln Pro Leu 130 135 140

Lys Arg Ser Val Ser Ala Gly Leu Tyr Met Ser Pro Gly Ser Pro Ser 145 150 155

Gly Ser Asp Val Ser Asp Ser Ser Val Pro Val Leu Ser Ser Ser Tyr 165 170 175

Val Tyr Lys Pro Ile Pro Arg Thr Gly Gly Val Asn Val Asn 180 185 190

Val Thr Pro Ala Gly Val Glu Ala Ala Ser Ser Ser Asn Asp Pro Pro 195 200 205

Thr Ser Leu Ser Leu Ser Leu Pro Gly Val Glu Ser Cys Glu Val Val 210 215 220

Ser Thr Gln Pro Ile Thr Glu Ser Thr Gln Asn Arg Ser Glu Glu Arg 225 230 235 240

Gly Gly Gly Val Met Gly Phe Ser Ala Glu Phe Met Ala Val Met Gln 245 250 255 Glu Met Ile Arg Val Glu Val Arg Asn Tyr Met Thr Gln Met Gln Gln

Gln Gln Gln Gln Asn Gly Ala Val Pro Gly Gly Ala Gly Met Gly 280

Met Cys Leu Asp Gly Gly Phe Arg Asn Leu Met Ala Val Asn Pro Val

Gly Met Ser Lys Ile Glu

<210> 115 <211> 593

<212> PRT

<213> Gossypium hirsutum

<400> 115

Met Gly Gly Pro Pro Tyr Asp Cys Leu Ala Asn Pro Leu Gly Ala Val

Arg Leu Thr Phe Glu Lys Ala Ile Trp Ser Glu Ser Glu Thr Pro Pro

Ile His Pro Ser Ala Phe Asn Gly Lys Asp Trp Gly Ala Leu Glu Leu

Phe Arg His Phe Leu Phe Gln Gly Ser Gly Leu Ser Gln Val Pro Ile

Leu Asn Pro Lys Thr Leu Arg Trp Val Gln Pro Asn Ser Leu Val Arg

Tyr Arg Gly Met Ile Gln Asp Met Leu Gly Asn Glu Phe Tyr Ala Gly

Ala Tyr Lys Asp Gly Asn Leu Trp Arg Thr Asn Lys Phe Met Asp Val

Ser Gln Tyr Pro Met Gly Ser Ser Pro Asp Met Cys Ile Trp Glu Arg

Arg Leu Leu Tyr Cys Val Pro Val Pro Gly Gln Asn Ser Trp Thr Glu

Pro Ser Ser Glu Met Glu Pro Asn Trp Ser Ser Gln Thr Arg Glu Lys

Arg Arg Arg Met Asp Asp Glu Asp Asn Asp Pro Met Asp Leu Val Pro

Asp Asp Glu Ile Lys Ser Ser Pro Ile Thr Lys Lys Met Arg Glu Asp

Gly Leu Pro Ser Pro Ser Gln Ser Arg Asp Thr Lys Thr Thr Ser Ser

- Ser Ser Ile Thr Ser Thr Phe Gln Ser Val Asp Glu Asp Asn Leu Pro 210 215 220
- Cys Leu Val Lys Ile Tyr Asp Ser Pro Glu Ser Glu Leu Lys Leu Asn 225 230 235 240
- Asp Val Phe Glu Phe Ile Gly Val Leu Thr Phe Asp Ser Glu Leu Ala 245 250 255
- Val Glu Lys Asp Asp Asn Asp Glu Leu Ser Asn Ser Phe Tyr Asp Asp 260 265 270
- Ala Leu Val His Leu Pro Pro Asn Lys Val Pro Arg Leu His Cys Leu 275 280 285
- Ile His Arg Lys Leu Ala Val Gln Asp Phe Leu Pro Gly Ser Pro Ile 290 295 300
- Ile Glu Pro Lys Pro His Leu Val Lys Glu Thr Arg Glu Ala Leu Phe 305 310 315 320
- Arg His Leu Thr Ala Val Leu Gly Asn Asp Glu Val Ala Ala His Phe 325 330 335
- Val Leu Leu His Leu Leu Ser Lys Val His Ala Arg Val Asp Asp Val 340 345 350
- Ala Val Gly Lys Leu Ser Leu Asn Leu Thr Gly Leu Asn Lys Glu Ser 355 360 365
- Val Ser Val Phe Gly Thr Arg Leu Ser Asp Thr Phe Lys Asn Leu Leu 370 375 380
  - Pro Phe Thr Asn Cys Met Pro Leu Thr Leu Glu Tyr Leu Asn Ile Ala. 385 390 400
  - Ser Leu Ala Pro Gln Lys Asp Tyr Gln Ala Asn Arg Leu Val Pro Gly
    405 410 415
  - Val Leu Gln Leu Pro Glu Gly Ser His Leu Met Val Asp Glu Thr Arg
    420 425 430
  - Leu Glu Ser Gly Ser Leu Asn Ser Thr Gly Ile Glu Asn Thr Lys Leu
    435 440 445
  - Leu Lys Asn Leu Ile Glu Phe Gln Lys Val Glu Tyr Asp Phe Gln Tyr 450 460
  - Tyr Lys Val Glu Met Ala Thr Asp Val Gln Leu Leu Ile Phe Ser Glu 465 470 475 480
  - Gly Lys Ser Asn Ile Val Pro Ala Asp Val Ile Val Pro Phe Gln Pro . 485 490 495
  - Ser Cys Leu Glu Ser Thr Glu Met Pro Val Ala Glu Ala Leu Glu Ala 500 505 510
  - Trp Arg Trp Tyr Leu Ala Thr Val Arg Ser Leu Pro His Ser Ile Gly

525 520

Ser Glu Ile Gln Lys Val Val Glu Asp Asp Leu Val Ala Ala Arg Gln

Met Asp Arg Ser Leu Gly Ser Arg Asp Phe Ser Arg Trp Leu Thr Met

Ala Arg Leu Ile Ser Ser Ser Phe Gly Glu Thr Ser Leu Ser Lys Glu

His Trp Glu Met Ala Lys Glu Met Glu Arg Leu Arg Arg Glu Arg Leu 585

Lys

<210> 116

<211> 89

<212> PRT

<213> Gossypium hirsutum

<220>

<221> misc\_feature

<222> (48)..(48)

<223> Xaa can be any naturally occurring amino acid

<400> 116

Met Ser Met Lys Lys Glu Gly Glu Ile Leu Tyr Lys Lys Gly Leu Trp

Ala Met Glu Glu Asp Lys Leu Leu Ile Asp Tyr Val Asn Val His Gly

Lys Gly Gln-Trp Asn Lys Ile Ala Asn Arg. Thr. Gly Leu Lys Arg Xaa

Gly Lys Ser Cys Arg Leu Arg Trp Met Asn Tyr Leu Ser Pro Asn Val

Lys Lys Gly Asp Phe Ser Glu Glu Glu Glu Asp Leu Val Ile Arg Leu 75

His Lys Leu Leu Glu Thr Gly Gly Leu 85

<210> 117

<211> 628 <212> PRT

<213> Gossypium hirsutum

<400> 117

Met Ser Thr Gly Val Gln His Gln Glu Arg Val Pro Met Asn Leu Lys

Lys Gln Leu Ala Leu Ala Val Arg Asn Ile Gln Trp Ser Tyr Ala Ile

Phe Trp Ser Ile Ser Thr Arg Gln Pro Gly Val Leu Glu Trp Gly Glu 40

- Gly Tyr Tyr Asn Gly Asp Ile Lys Thr Arg Lys Thr Val Gln Ser Val  $50\,$
- Glu Leu Asn Thr Asp Gln Leu Ser Leu Gln Arg Ser Glu Gln Leu Arg 65 70 75 80
- Gln Leu Tyr Glu Ser Leu Ser Ala Gly Glu Ser Ser Pro Gln Ala Lys 85 90 95
- Arg Pro Ser Ala Ala Leu Ser Pro Glu Asp Leu Thr Asp Thr Glu Trp
- Tyr Tyr Leu Val Cys Met Ser Phe Val Phe Asn Ile Gly Gln Gly Leu 115 120 125
- Pro Gly Arg Thr Leu Ser Thr Gly Gln Pro Val Trp Leu Cys Asn Ala 130 135 140
- His Cys Ala Asp Ser Lys Val Phe Gly Arg Ser Leu Leu Ala Lys Ser 145 150 155 160
- Ala Ser Ile Gln Thr Ala Val Cys Phe Pro Phe Ser Gly Gly Val Val
  165 170 175
- Glu Leu Gly Val Thr Asp Leu Val Phe Glu Asp Leu Ser Leu Ile Gln 180 185 190
- Arg Val Lys Thr Leu Leu Leu Asp Asp Pro Gln Pro Ile Val Ser Lys 195 200 205
- Arg Ser Ile Gln Val Asp Gly Met Asn Asn Asp Leu Ala Cys Pro Ala 210. 215 220
- Leu Asp Pro Leu Ile Leu Ala Thr Lys Leu Ser Pro Ile Leu Gly Cys 225 · 230 235 240
- Glu Gln Leu Glu Thr Val Ser Pro Asp Asp Ser Pro Asp Gly Leu Glu 245 250 255
- Pro Lys Gln Ser Arg Glu Asp Ser Leu Leu Ile Glu Gly Ile Asn Gly 260 265 270
- Gly Ala Ser Gln Val Gln Ser Trp Gln Phe Met Asp Glu Glu Phe Cys 275 280 285
- Asn Cys Val His His Ser Leu Asn Ser Ser Asp Cys Ile Ser Gln Thr 290 · - 295 300
- Ile Ala Asp His Arg Lys Val Val Pro Leu Tyr Arg Gly Glu Asn Asp 305 310 315 320
- Asn Gly Leu Gln Asp Val Glu Glu Cys Asn Gln Thr Lys Leu Thr Ser
- Phe Asp Arg Gln Asn Asp Asp Arg His Phe His Glu Val Leu Ser Ala 340 345 350 .

Leu Phe Lys Ser Ser His Pro Leu Ile Leu Gly Pro Gln Phe Arg Asn

Ser Asn Lys Glu Ser Ser Phe Ile Arg Trp Gln Lys Asn Gly Leu Val

Lys Pro Gln Lys Glu Arg Asp Glu Thr Pro Gln Lys Leu Leu Lys Lys

Ile Leu Phe Leu Val Pro His Met His Asp Arg Gly Leu Ile Glu Ser

Pro Glu Thr Asn Ala Val Arg Asp Ala Ala Trp Arg Pro Glu Ala Asp 425

Glu Ile Cys Gly Asn His Val Leu Ser Glu Arg Lys Arg Arg Glu Lys 440

Ile Asn Glu Arg Leu Met Met Leu Lys Ser Leu Val Pro Ala Asn Asn

Lys Ala Asp Lys Val Ser Ile Leu Asp Val Thr Ile Glu Tyr Leu Gln

Thr Leu Glu Arg Arg Val Ala Glu Leu Glu Ser Cys Arg Lys Ser Glu

Ala Arg Thr Lys Ile Glu Arg Thr Ser Asp Asn Tyr Gly Asn Asn Lys

Thr Asn Asn Gly Lys Lys Ser Ser Leu Ser Lys Arg Lys Ala Tyr Asp 520

Val Val Asp Glu Ala Asp Gln Glu Ile Gly Tyr Val Ala Ser Lys Asp

Gly Ser Thr Asp Lys Val Thr Leu Ser Met Asn Asn Lys Glu Leu Leu 545 550 560

Ile Glu Phe Lys Cys Pro Trp Arg Glu Gly Ile Leu Leu Glu Val Met

Asp Ala Leu Ser Ile Leu Asn Leu Asp Cys His Ser Val Gln Ser Ser

Thr Thr Glu Gly Ile Leu Ser Leu Thr Ile Lys Ser Lys Tyr Lys Gly

Ser Ser Val Ala Lys Ala Gly Pro Ile Glu Gln Ala Leu Gln Arg Ile 615

Ala Ser Lys Cys 625

<210> 118 <211> 123

<212> PRT

<213> Gossypium hirsutum

<400> 118

Met Ala Ser Ser Gly Val Leu Lys Leu Val Ser Met Ile Leu Met Val

Cys Met Thr Met Met Ser Ala Pro Lys Ala Ala Lys Ala Ala Ile Thr  $20 \hspace{1cm} 25 \hspace{1cm} 30$ 

Cys Ser Asp Val Val Asn His Leu Ile Pro Cys Leu Ser Tyr Val Gln

Asn Gly Gly Thr Pro Ala Ala Ala Cys Cys Ser Gly Val Lys Ala Leu

Tyr Gly Glu Val Gln Thr Ser Pro Asp Arg Gln Asn Val Cys Lys Cys

Ile Lys Ser Ala Val Asn Gly Ile Pro Tyr Thr Ser Asn Asn Leu Asn

Leu Ala Ala Gly Leu Pro Ala Lys Cys Gly Leu Gln Leu Pro Tyr Ser

Ile Ser. Pro Ser Thr Asp Cys Asn Lys Val Gln

<210> 119

<211> 362 <212> PRT

<213> Gossypium hirsutum

<400> 119

Met Ala Asn His Thr Val Thr Phe Leu Pro Lys Leu Ser Ile Glu Ala

Ile Gln Thr Val Thr Pro Met Arg Ile Thr Glu Pro Arg Gln Thr Arg

Gln Val Leu Ala Gly Glu Leu Val Gly Pro Gly Ile Phe Gln Arg Cys

Leu Asn Val Val Gln Tyr Tyr Met Lys Glu Lys Glu Glu Asp Ser Gly

Trp Leu Leu Ala Gly Trp Ile Lys Glu Thr Leu Gly Arg Ala Leu His

Glu Gln Pro Met Ile Ser Gly Arg Leu Arg Lys Gly Glu Arg Asn Asp

Gly Glu Leu Glu Ile Val Ser Asn Asp Cys Gly Ile Arg Leu Ile Glu

Ala Arg Ile Gln Met Asn Leu Ser Asp Phe Leu Asp Leu Lys Gln Arg

Glu Asp Ala Glu Ala Gln Leu Val Phe Trp Lys Asp Ile Asp Glu Gln

Asn Pro Gln Phe Ser Pro Leu Phe Tyr Val Gln Val Thr Asn Phe Gln

Cys Gly Gly Tyr Ser Ile Gly Ile Ser Cys Ser Ile Leu Leu Ala Asp

Leu Leu Leu Met Lys Glu Phe Leu Lys Thr Trp Ala Asp Ile His Asn

Lys Val Ile Ile Asn Lys Asn Asp Glu Gln Lys Leu Pro Leu Phe Tyr

Leu Pro Gly Leu Lys Asn Thr Asn Gly Ala Ser Pro Asn Ile Ile Thr 215

Ser Asn Ser Ser Lys Asn Ser Ala Lys Thr Met Ile Phe Gln Ile Gln 230

Ala Glu Thr Glu Ser Pro Gly Ser Asp Trp Cys Arg Lys Met Ala Leu 250

Ala Cys Leu Glu Glu Ala Glu Ser Asn Leu Gly Ser Val Val Gly Gly 265

Glu Phe Ser Leu Phe Val Asn Glu Ser Phe Glu Ser Ile Lys Val Glu 280

Ser Cys Ser Lys Gln Gly Met Ser Lys Glu Ala Glu Met Gly Val Leu

Asn Arg Ala Lys Trp Asp Asp Leu Gly Ala Asn Glu Val Ser Phe Gly 310

Asp Gly Asn Lys Pro Ala His Val Ser Tyr Trp Leu Arg Ser Thr Leu

Gly Gly Leu Ile Ile Val Ile Pro Ser Leu Gln Glu Asp Lys Tyr Thr 345

Val Asn Ile Ile Val Thr Ile Pro Ser Lys 360

<210> 120 . <211> 497

<212> PRT

<213> Gossypium hirsutum

<400> 120

Met Gly Phe Gln Arg Asn Ile Leu Gly Phe Leu Leu Leu Ile Leu Ala 10

Ser Leu Thr Ser Leu Ser Ser Ser Leu Pro Ser Glu Tyr Ser Ile Val

Glu His Glu Ile Asp Ala Phe Leu Ser Glu Glu Arg Val Leu Glu Ile

Phe Gln Gln Trp Lys Glu Lys Asn Gln Lys Val Tyr Arg Gln Ala Glu

50 55 60

Glu Ala Glu Lys Arg Phe Glu Asn Phe Lys Gly Asn Leu Lys Tyr Ile
70 75 80

Leu Glu Arg Asn Ala Lys Arg Lys Ala Asn Lys Trp Glu His His Val 85 90 95

Gly Leu Asn Lys Phe Ala Asp Met Ser Asn Glu Glu Phe Arg Lys Ala 100 105 110

Tyr Leu Ser Lys Val Lys Lys Pro Ile Asn Lys Gly Ile Thr Leu Ser

Arg Asn Met Arg Arg Lys Val Gln Ser Cys Asp Ala Pro Ser Ser Leu 130 135 140

Asn Trp Arg Asn Tyr Gly Val Val Thr Ala Val Lys Asp Gln Gly Ser 145 150 155 160

Cys Gly Ser Cys Trp Ala Phe Ser Ser Thr Gly Ala Met Glu Gly Ile 165 170 175

Asn Ala Leu Val Thr Gly Asp Leu Ile Ser Leu Ser Glu Gln Glu Leu 180 185 190

Val Asp Cys Asp Thr Ser Asn Tyr Gly Cys Glu Gly Gly Tyr Met Asp 195 200 205

Tyr Ala Phe Glu Trp Val Ile Asn Asn Gly Gly Ile Asp Ser Glu Thr 210 215 220

Glu Thr Lys Val Val Ser Ile Asp Gly Tyr Gln Asp Val Glu Gln Ser 245 250 255

Asp Ser Ala Leu Leu Cys Ala Val Ala Gln Gln Pro Val Ser Val Gly 260 265 270

Ile Asp Gly Ser Ala Ile Asp Phe Gln Leu Tyr Thr Gly Gly Ile Tyr 275 280 285

Asp Gly Ser Cys Ser Asp Asp Pro Asp Asp Ile Asp His Ala Val Leu

Ile Val Gly Tyr Gly Ser Glu Gly Ser Glu Glu Tyr Trp Ile Val Lys 305 310 315 320

Asn Ser Trp Gly Thr Ser Trp Gly Ile Asp Gly Tyr Phe Tyr Leu Lys 325 330 335

Ser Tyr Pro Thr Lys Glu Ser Ser Ser Pro Ser Pro Tyr Pro Ser Pro 355 360 365

Ser Val Pro Pro Pro Pro Pro Ser Thr Pro Pro Pro Pro Pro Pro

Pro Ser Pro Ser Pro Ser Asp Cys Gly Asp Phe Ser Tyr Cys Ser Ser

Asp Glu Thr Cys Cys Cys Leu Phe Glu Phe Tyr Asp Tyr Cys Leu Ile

Tyr Gly Cys Cys Glu Tyr Glu Asn Ala Val Cys Cys Thr Gly Thr Glu

Tyr Cys Cys Pro Ser Asp Tyr Pro Ile Cys Asp Val Gln Glu Gly Leu

Cys Leu Lys Asn Ala Gly Asp Tyr Leu Gly Val Ala Ala Arg Lys Arg

Lys Val Ala Lys His Lys Leu Pro Trp Thr Lys Ile Glu Glu Thr Glu

Ile Thr Tyr Gln Pro Leu Gln Trp Lys Arg Asn Pro Phe Ala Ala Met

Arg

<210> 121

<211> 335 <212> PRT

<213> Gossypium hirsutum

<400> 121

Met Lys Val Leu Ser Pro Ile Leu Ala Cys Leu Ala Leu Ala Val Val

Val Ser His Ala Ala Leu Ser Pro Glu Gln Tyr Trp Ser Tyr Lys Leu 20

Pro Asn Thr Pro Met Pro Lys Ala Val Lys Glu Ile Leu His Pro Glu

Leu Met Glu Glu Lys Ser Thr Ser Val Asn Val Gly Gly Gly Val

Asn Val Asn Thr Gly Lys Gly Lys Pro Gly Gly Asp Thr His Val Asn 65 70 75 80

Val Gly Gly Lys Gly Val Gly Val Asn Thr Gly Lys Pro Gly Gly Gly

. Thr His Val Asn Val Gly Asp Pro Phe Asn Tyr Leu Tyr Ala Ala Ser

Glu Thr Gln Ile His Glu Asp Pro Asn Val Ala Leu Phe Phe Leu Glu 120

Lys Asp Met His Pro Gly Ala Thr Met Ser Leu His Phe Thr Glu Asn

Thr Glu Lys Ser Ala Phe Leu Pro Tyr Gln Thr Ala Gln Lys Ile Pro

Phe Ser Ser Asp Lys Leu Pro Glu Ile Phe Asn Lys Phe Ser Val Lys

Pro Gly Ser Val Lys Ala Glu Met Met Lys Asn Thr Ile Lys Glu Cys 185

Glu Gln Pro Ala Ile Glu Gly Glu Glu Lys Tyr Cys Ala Thr Ser Leu 200

Glu Ser Met Ile Asp Tyr Ser Ile Ser Lys Leu Gly Lys Val Asp Gln 215

Ala Val Ser Thr Glu Val Glu Lys Gln Thr Pro Met Gln Lys Tyr Thr

Ile Ala Ala Gly Val Gln Lys Met Thr Asp Asp Lys Ala Val Val Cys 250

His Lys Gln Asn Tyr Ala Tyr Ala Val Phe Tyr Cys His Lys Ser Glu

Thr Thr Arg Ala Tyr Met Val Pro Leu Glu Gly Ala Asp Gly Thr Lys 280

Ala Lys Ala Val Ala Val Cys His Thr Asp Thr Ser Ala Trp Asn Pro

·Lys His Leu Ala Phe Gln Val Leu Lys Val Glu Pro Gly Thr Ile Pro . 305 310

Val Cys His Phe Leu Pro Arg Asp His Ile Val Trp Val Pro Lys 325 330

<210> 122 <211> 302 <212> PRT

<213> Gossypium hirsutum

<400> 122

Met Glu Arg Gln Arg Ser Lys Gln Val Cys Leu Leu Met Trp Val Leu

Val Ala Ala Phe Phe Ser His Asn Arg Val Ile Ala Val Thr Ser Thr

Gly Leu Gly Glu Gln Lys Asn Tyr Tyr Pro Ala Pro Asp Pro His Ala

Gly Thr Pro Pro Ser Gly Ser His Gly Thr Pro Pro Ser Ser Gly Gly

Gly Ser Pro Pro Ser His Gly Thr Pro Ser His Gly Gly Tyr His 75

Pro Ser Pro Thr Pro Ser Thr Pro Ser Gly Gly Asn Cys Gly Thr Pro 85 90

Pro His Asp Pro Ser Thr Pro Ser Thr Pro Ser His Thr Pro Pro His 100 105 110

Gly Thr Pro Pro Ser Ser Gly Gly Gly Ser Pro Pro Ser Tyr Gly Gly
115 120 125

Gly Ser Pro Pro Ser Tyr Gly Gly Gly Ser Pro Pro Ser Tyr Gly Gly 130 135 140

Gly Ser Pro Pro Ser Tyr Gly Gly Gly Ser Pro Pro Ser Tyr Gly Gly 145 150 155 160

Gly Ser Pro Pro Thr Thr Pro Ile Asp Pro Gly Thr Pro Ser Ile Pro 165 170 175

Ser Pro Pro Phe Phe Pro Ala Pro Thr Pro Pro Ile Gly Gly Thr Cys 180 185 190

Asp Phe Trp Arg Ser His Pro Thr Leu Ile Trp Gly Leu Leu Gly Trp 195 200 205

Trp Gly Thr Val Gly Asn Ala Phe Gly Val Thr Asn Ala Pro Gly Leu 210 215 220

Gly Thr Ser Met Ser Leu Pro Gln Ala Leu Ser Asn Thr Arg Thr Asp 225 230 235 240

Gly Leu Gly Ala Leu Tyr Arg Glu Gly Thr Ala Ser Phe Leu Asn Ser 245 250 255

Met Val Asn Asn Arg Phe Pro Phe Ser Thr Lys Gln Val Arg Glu Thr

Phe Val Ala Ala Leu Gly Ser Asn Ser Ala Ala Ala Ala Gln Ala Arg 275 280 285

Leu Phe Lys Leu Ala Asn Glu Gly His Leu Lys Pro Arg Thr

<210> 123

<211> 196

<212> PRT

<213> Gossypium hirsutum

<400> 123

Met Met Lys Arg Gly Phe Ile Val Leu Ala Leu Thr Val Val Phe Ala 1 5 10 15

Ala Thr Val Val Thr Ala Ala Asp Glu Ser Gly Leu Ala Asn Glu Cys 20 25 30

Ser Lys Asp Phe Gln Ser Val Met Thr Cys Leu Ser Phe Ala Gln Gly

Lys Ala Ala Ser Pro Ser Lys Glu Cys Cys Asn Ser Val Ala Gly Ile 50 55 60

Lys Glu Asn Lys Pro Lys Cys Leu Cys Tyr Ile Leu Gln Gln Thr Gln 65 70 75 80

Thr Ser Gly Ala Gln Asn Leu Lys Ser Leu Gly Val Gln Glu Asp Lys 85 90 95

Leu Phe Gln Leu Pro Ser Ala Cys Gln Leu Lys Asn Ala Ser Val Ser 100 105 110

Asp Cys Pro Lys Leu Leu Gly Leu Ser Pro Ser Ser Pro Asp Ala Ala 115 120 125

Ile Phe Thr Asn Ser Ser Ser Lys Ala Thr Thr Pro Ser Thr Ser Thr 130 135 140

Thr Thr Ala Thr Pro Ser Ser Ala Ala Asp Lys Thr Asp Ser Lys Ser 145 155 160

Ser Gly Ile Lys Leu Gly Pro His Phe Val Gly Ser Thr Ala Ala Leu 165 170 175

Leu Val Ala Thr Ala Ala Val Phe Phe Leu Val Phe Pro Ala Gly Phe
180 185 190

Ala Ser Ile Val 195

<210> 124

<211> 629

<212> PRT

<213> Gossypium hirsutum

<400> 124

Met Pro Val Val Asp Phe Ala Cys Val Phe Leu Val Ser Val Val Met
1 5 10 15

Phe Asn Leu Arg Val Ser Thr Glu Pro Val Glu Asp Lys Gln Ala Leu 20 25 30

Leu Ala Phe Ile Ser Gly Ile Arg His Ala Asp Arg Val Lys Trp Asn 35 40 45

Ser Ser Thr Ser Ala Cys Asp Trp Phe Gly Val Gln Cys Asp Ala Asn 50 55 60

Arg Ser Phe Val Tyr Thr Leu Arg Val Pro Gly Trp Gly Pro Tyr Gly 65 75 80

Val Arg Phe Arg Pro Lys Gln Ile Gly Arg Leu Asn Arg Leu Arg Val 85 90 95

Leu Ser Leu Arg Ala Asn Arg Leu Ser Gly Glu Ile Pro Ala Asp Phe 100 105 110

Tyr Asn Leu Thr Gln Leu Arg Ser Leu Tyr Leu Gln Gly Asn Glu Phe 115 120 125 Thr Gly Pro Phe Pro Pro Ser Val Thr Arg Leu Thr Arg Leu Thr Arg 130 135 140

- Leu Asp Leu Ser Ser Asn Asn Phe Thr Gly Pro Ile Pro Leu Gly Val 145 150 155 160
- Asn Asn Leu Thr Gln Leu Thr Lys Leu Phe Leu Gln Asn Asn Lys Phe 165 170 175
- Ser Gly Ser Leu Pro Ser Ile Asp Ser Asp Gly Leu Asn Asp Phe Asn 180 185 190
- Val Ser Asn Asn Leu Lys Gly Ser Ile Pro Asp Ser Leu Ser Lys 195 200 205
- Phe Pro Glu Ser Ser Phe Ala Gly Asn Ile Gly Leu Cys Gly Gly Pro 210 215 220
- Leu Arg Pro Cys Asn Pro Phe Pro Pro Ser Pro Ser Pro Thr Glu Pro 225 230 235 240
- Ile Pro Pro Lys Thr Ser Gly Gln Ser Ser Lys Ser Leu Pro Thr Gly
  245 250 255
- Ala Ile Ile Ala Ile Ala Val Gly Ser Ala Ile Val Ala Leu Leu Leu 260 265 270
- Leu Leu Phe Leu Île Île Cys Phe Arg Lys Trp Lys Arg Lys Ser Pro 275 280 285
- Arg Arg Gln Lys Ala Ile Pro Ser Thr Thr His Ala Leu Pro Val Glu 290 . 295 300
- Glu Ala Gly Thr Ser Ser Ser Lys Asp Asp Ile Thr Gly Gly Ser Thr 305 310 315
- Glu Ile Glu Arg Met Met Asn Asn Lys Leu Met Phe Phe Lys Gly Gly 325 330 335
- Val Tyr Ser Phe Asp Leu Glu Asp Leu Met Arg Ala Ser Ala Glu Met 340 345 350
- Leu Gly Lys Gly Ser Thr Gly Thr Ser Tyr Arg Val Val Leu Ala Val 355 365
- Gly Thr Thr Val Ala Val Lys Arg Leu Lys Asp Val Ala Val Ser Lys 370 375 380
- Arg Glu Phe Val Met Lys Met Gly Met Leu Gly Lys Ile Met His Glu 385 390 395 400
- Asn Val Val Pro Leu Arg Ala Phe Tyr Tyr Ser Asp Glu Glu Lys Leu 405 410 415
- Leu Val Tyr Asp Tyr Met His Gly Gly Ser Leu Phe Ala Leu Leu His 420 425 430

Gly Ser Arg Ser Ser Ala Arg Thr Pro Leu Glu Trp Asp Pro Arg Met

Lys Ile Ala Leu Gly Val Ala Arg Gly Leu Ala His Leu His Ser Ser 450 460

Gln Asn Met Val His Gly Asn Ile Lys Ser Ser Asn Ile Leu Leu Arg 465 470 475 480

Pro Asp His Glu Ala Cys Ile Ser Glu Phe Gly Leu Asn Ser Leu Phe 485 490 495

Asn Thr Asn Thr Pro Pro Ser Arg Ile Ala Gly Tyr Gln Ala Pro Glu 500 505 510

Val Ile Gln Thr His Lys Val Thr Val Lys Ser Asp Val Tyr Ser Phe 515 520 525

Gly Val Leu Leu Glu Leu Leu Thr Gly Arg Ala Pro Ile Gln Pro 530 535 540

Ser Ile Thr Glu Glu Gly Phe Asp Leu Pro Arg Trp Val Gln Ser Val 545 550 555 560

Val Arg Glu Glu Trp Ala Ala Glu Val Phe Asp Ala Glu Leu Met Ala 565 570 575

Tyr His Asp Ile Glu Glu Glu Met Val Gln Ala Leu Gln Met Ala Met 580 590

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35 40 45

Leu Lys Asn Asp Arg Val Val Met Tyr Asp Arg Thr Asp Phe Gly Pro 50 60

Ser Thr Leu Pro Leu Ala Ser Gly Lys Cys His Asn Asp Pro Thr Asn

80 75 70 65 Thr Ala Val Gln Val Asp Cys Thr Ala His Ser Val Glu Tyr Asp Val Leu Ser Asn Lys Phe Arg Ala Leu Thr Val Gln Ser Asn Val Trp Cys Ser Ser Gly Gly Val Met Pro Asp Gly Lys Leu Val Gln Thr Gly Gly 120 Phe Ser Glu Gly Glu Leu Arg Val Arg Val Phe Ser Pro Cys Glu Ser Cys Asp Trp His Glu Thr Pro Asn Gly Leu Ala Ala Lys Arg Trp Tyr Ala Thr Asn His Val Leu Pro Asp Gly Arg Gln Ile Val Val Gly Gly Arg Glu Gln Phe Asn Tyr Glu Phe Val Pro Lys Asn Ile Ala Ala Asp Thr Phe Lys Leu His Phe Leu Ser Glu Thr Asn Glu Arg Gly Val Glu Asn Asn Leu Tyr Pro Phe Val Phe Leu Asn Val Asp Gly Asn Leu Phe Ile Phe Ala Asn Asn Arg Ala Ile Leu Leu Asp Tyr Val Asn Asn Lys 230 Val Val Lys Thr Tyr Pro Lys Ile Pro Gly Gly Glu Pro Arg Ser Tyr Pro Ser Thr Gly Ser Ala Val Leu Leu Pro Leu Lys Asn Leu Thr Ala 260 Ala Thr Ile Gln Ala Glu Val Leu Val Cys Gly Gly Ala Pro Lys Gly Ser Phe Val Gln Ala Leu Gln Gly Lys Phe Val Lys Ala Leu Asn Thr Cys Ala Arg Ile Ser Ile Thr Asp Pro Lys Pro Lys Trp Val Leu Glu Thr Met Pro Leu Ala Arg Val Met Gly Asp Met Val Leu Leu Pro Asn 330 Gly Lys Val Leu Val Ile Asn Gly Ala Arg Ser Gly Ser Ala Gly Trp

Asn Glu Ile Glu Ser Arg Phe Lys Ile Leu Asn Pro Thr Lys Ile Pro 370 375 380

Asp Leu Gly Arg Asp Pro Val Leu Asn Pro Val Leu Tyr Met Pro Asp

Arg Met Tyr His Ser Thr Ala Val Leu Leu Arg Asp Gly Arg Val Leu 385 390 395 400

Val Gly Gly Ser Asn Pro His Ala Tyr Tyr Asn Phe Thr Gly Val Leu 405 410 415

Týr Pro Thr Glu Leu Ser Leu Glu Ala Phe Tyr Pro Gly Tyr Leu Asp 420 425 430

Ala Lys Phe Asn Asn Leu Arg Pro Thr Ile Val Ala Pro Lys Ser Met 435 440 445

Ser Gly Ile Arg Tyr Asn Lys Lys Leu Lys Ile Lys Val Val Ile Thr 450 455 460

Gly Glu Val Thr Leu Asn Leu Leu Ser Val Thr Met Val Ser Pro Ala 465 470 475 480

Phe Asn Thr His Ser Phe Ser Met Asn Gln Arg Leu Leu Val Leu Gly 485 495

Asn Asp Lys Val Met Ala Ser Gly Lys Ser Thr Tyr Glu Ile Glu Val 500 505 510

Met Thr Pro Gly Ser Gly Asn Leu Ala Pro Ala Gly Phe Tyr Leu Leu 515 520 525

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Glu Ile Ser Gln Ala Phe Ser Gly Leu Arg Val Ser Ile Asp Cys Lys 50 55 60

Pro Glu Asn Gly Lys Asn Phe Lys Thr Arg Gly Ser Gly Glu Leu Asp 65 75 80

Lys Gln Gly Asn Phe Lys Val Phe Val Pro Glu Asp Leu Val Glu Asn 85 90 95

- Gly Glu Leu Lys Glu Glu Cys Tyr Ala Gln Leu His Ser Val Ser Ala 100 105 110
- Ala Pro Cys Pro Ala His Asp Gly Leu Glu Ser Ala Lys Leu Val Leu 115 120 125
- Lys Ser Arg Ser Asp Gly Lys His Gly Phe Gly Leu Lys Gly Lys Leu 130 135 140
- Arg Phe Ser Pro Leu Thr Cys Ala Ser Ala Phe Phe Trp Pro His Phe 145 150 160
- Lys Phe Pro Pro Leu Pro Lys Trp Asn His Pro Pro Leu Pro Lys Phe 165 170 175
- Pro Leu Pro Pro Phe Lys Gly Phe His His His Tyr Pro Ile Ile Pro 180 185 190
- Pro Ile Tyr Lys Lys Pro Leu Pro Pro Pro Ser Pro Val Tyr Lys Pro 195 200 205
- Pro Pro Val Pro Val Asn Pro Pro Val Pro Ile Tyr Lys Pro Pro Pro 210 215 220
- Val Pro Val Tyr Lys Pro Pro Pro Val Pro Val Lys Pro Leu Pro Pro 225 230 235 240
- Pro Val Pro Ile Tyr Lys Pro Pro Pro Val Glu Lys Pro His Pro Pro 245 250 255
- Pro Val Pro Val Tyr Lys Pro Pro Val Pro Val Tyr Lys Lys Pro
  260 265 270
- Cys Pro Pro Pro Val Pro Val Tyr Lys Ser Pro Pro Val Pro Val Tyr 280 285
- Lys Lys Pro His Pro Pro Pro Val Pro Val Tyr Lys Lys Pro His Pro 290 295 300
- Pro Pro Val Pro Val Tyr Lys Lys Pro Cys Pro Pro Pro Val Pro Val 305 310 315 320
- Tyr Lys Ser Pro Pro Val Pro Glu Pro His Pro Pro Pro Val Pro Val 325 330 335
- Tyr Lys Lys Pro His Pro Pro Pro Val Pro Val Tyr Lys Lys Pro Cys 340 345 350
- Pro Pro Pro Val Pro Val Tyr Lys Ser Pro Pro Val Pro Glu Pro His 355 360 365
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- Val Tyr Asn Lys Pro Leu Pro Pro Pro Val Pro Val Tyr Thr Lys Pro

405 410 415

Leu Pro Pro Pro Val Pro Thr Tyr Lys Pro Lys Pro Leu Pro Pro Ile 420 425 430

Pro Tyr Lys Pro Leu Pro Pro Leu Pro Lys Ile Pro Pro Phe Pro Lys 435

Lys Pro Cys Pro Pro Leu Pro Lys Leu Pro Pro Leu Pro Lys Ile Pro 450 450 455

Pro Lys Tyr Phe His His His Pro Pro Leu Pro Lys Leu Pro Pro Leu 465 470 475 480

Pro Lys Ile Pro Pro Lys Tyr Phe His His His Pro Lys Phe Gly Lys 485 490 495

Trp Pro Ser Leu Pro Pro Phe Ala Pro His His Pro 500 505

## (19) World Intellectual Property Organization International Bureau





(43) International Publication Date 22 December 2005 (22.12.2005)

T (10) Inter

## (10) International Publication Number WO 2005/121364 A3

(51) International Patent Classification: C12N 15/29 (2006.01) C12N 15/11 (2006.01)

(21) International Application Number:

PCT/IL2005/000627

(22) International Filing Date: 14 June 2005 (14.06.2005)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data: 60/578,833

14 June 2004 (14.06.2004) US

(71) Applicant (for all designated States except US): EVO-GENE LTD. [IL/IL]; 13 Gad Finstein Street, 76121 Rehovot (IL).

(72) Inventors; and

- (75) Inventors/Applicants (for US only): RONEN, Gil [IL/IL]; 20 Moshav Ometz, 33870 Emeq-Hefer (IL). GOLD, Evgenia [IL/IL]; 11/3 Yavne Street, 76360 Rechovot (IL). YELIN, Rodrigo [IL/IL]; 12 Odem Street, 44862 Zur-Yigal (IL). MEISSNER, Rafael [IL/IL]; 11 HaLevona Street, 76350 Rechovot (IL). KARCHI, Hagai [IL/IL]; 249 Sapir Street, Moshav Sitriya, 76834 Doar-Na Emek Soreq (IL). AYAL, Sharon [IL/IL]; 9 Ha-Shita, P.O Box 209, 76951 Kiryat-Ekron (IL).
- (74) Agent: G.E. EHRLICH (1995) LTD.; 11 Menachem Begin Street, 52 521 Ramat Gan (IL).

- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GO, GW, ML, MR, NE, SN, TD, TG).

#### Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report: 8 March 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYNUCLEOTIDES AND POLYPEPTIDES INVOLVED IN PLANT FIBER DEVELOPMENT AND METHODS OF USING SAME

(57) Abstract: Isolated polynucleotides are provided. Each of the isolated polynucleotides comprise a nucleic acid sequence encoding a polypeptide having an amino acid sequence at least 80 % homologous to SEQ ID NO: 26, 106, 107, 109, 110, 112, 114, 115, 118, 119, 122, 123, 124, 126, 95 or 96, wherein the polypeptide is capable of regulating cotton fiber development. Also provided are methods of using such polynucleotides for improving fiber quality and/or yield of a fiber producing plant, as well as methods of using such polynucleotides for producing plants having increased biomass/vigor/yield.



#### INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL05/00627

A. CLAS IPC:	SIFICATION OF SUBJECT MATTER C12N 15/29( 2007.01),15/11( 2007.01)						
USPC: According to	USPC: 536/23.6,24.3,23.1 According to International Patent Classification (IPC) or to both national classification and IPC						
B. FIELI	OS SEARCHED						
	cumentation searched (classification system followed b 36/23.6, 24.3, 23.1	y classification symbols)					
Documentatio	on searched other than minimum documentation to the	extent that such documents are included in	n the fields searched				
	ta base consulted during the international search (name EQ ID NOs: 26 and 74	e of data base and, where practicable, sear	ch terms used)				
C. DOC	UMENTS CONSIDERED TO BE RELEVANT						
Category *	Citation of document, with indication, where ap		Relevant to claim No.				
х	ORFORD, S.J. et al, Specific expression of an expainibres, Biochim. Biophys. Acta, 1998, Vol. 1398, pa	nsin gene during elongation of cotten	1, 3-6, and 10				
	Abstract and Figure 1 (page 343).	iges 342-340, see especially the	9				
Y	WALLACE, R.B. et al, Oligonucleotide probes for libraries, Methods Enzymol., 1987, Vol. 152, pages		9				
	·						
Further	documents are listed in the continuation of Box C.	See patent family annex.					
* S	pecial categories of cited documents:	"T" later document published after the inte date and not in conflict with the appli					
	defining the general state of the art which is not considered to be lar relevance	principle or theory underlying the inv	ention				
"E" earlier ap	plication or patent published on or after the international filing date	"X" document of particular relevance; the considered novel or cannot be conside when the document is taken alone	claimed invention cannot be cred to involve an inventive step				
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combinations.							
	referring to an oral disclosure, use, exhibition or other means	being obvious to a person skilled in the					
	"P" document published prior to the international filing date but later than the "&" document member of the same patent family priority date claimed						
l .	ctual completion of the international search	Date of mailing of the international sear	ch report				
	r 2006 (29.11.2006)	Authorized officer	U/				
	ailing address of the ISA/US il Stop PCT, Attn: ISA/US	James Marriagli of Rolling	t. 0-				
Cor	nmissioner for Patents b. Box 1450	Authorized officer  James Martinell  James Martinell	s 401				
Ale	Alexandria, Virginia 22313-1450  Facsimile No. (571) 273-3201  Telephone No. (571) 272-0719						

Facsimile No. (571) 273-3201
Form PCT/ISA/210 (second sheet) (April 2005)

### INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL05/00627

Box No.	Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)
This intern	ational search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1.	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2.	Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.	Claims Nos.: 15, 16, 20 and 23 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box No. 1	II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)
This Intern Please See	ational Searching Authority found multiple inventions in this international application, as follows:  Continuation Sheet
1.	As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. Remark on	No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: Please See Continuation Sheet  Protest  The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.  The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
	No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(2)) (April 2005)

# INTERNATIONAL SEARCH REPORT International application No. PCT/IL05/00627

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, claim(s) 1-14, drawn to polynucleotides and oligonucleotides.

Group II, claim(s) 17-19, 21, 22, 24-27, and 31, drawn to methods of regulating gene expression of combinations of no fewer than 16 genes.

Group III, claim(s) 28-30, drawn to methods of identifying genes.

Group IV, claim(s) 32, drawn to methods of producing cotton fibers by generating transgenic cotton plants expressing combinations of no fewer than 16 genes.

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons. The polynucleotides and oligonucleotides of Group I have the special technical features of the SEQ ID NOs listed in the claims of Group I. The methods of each one of Groups II and IV are drawn to the regulation and expression of combinations of no fewer than 16 genes for 65,535 different combinations, each combination having a set of special technical features that differs from the set of special technical features of each other combination. Group III is drawn to methods that do not require the use of any of the polynucleotides or oligonucleotides of Group I and so do not share the special technical features of Group I. The methods of Groups II, III, and IV may be practiced independently of one another and do not share the same special technical features.

Group I mentions or requires the use of a large number (16) of separate and unrelated nucleic acids. In the absence of payment of additional search fee(s) only the first mentioned SEQ ID NO in Group I (i.e., nucleic acids encoding SEQ ID NO: 26) will be searched. If applicant wishes more than one SEQ ID NO of Group I to be searched and examined, applicant is required to pay one additional search fee for each of the SEQ ID NOs applicant wishes to be searched and examined. Should applicant pay fee(s) for additional Groups to be searched, the SEQ ID NOs within the selected Group(s) will be searched in the order in which they appear in the claims unless applicant directs otherwise.

Each of Groups II and IV mentions or requires the use of a large number of combinations of polynucleotides. The simplest and first "combination" in Group II is SEQ ID NO: 17. A total of 65,535 combinations of the 16 SEQ ID NOs mentioned in each of Groups II and IV exists. Should applicant wish any of the combinations to be searched and examined, applicant is required to pay one additional search fee for each combination to be searched.

Continuation of Box III Item 4:

1-14 as they pertain to SEQ ID NO: 26, Thus claims 1-6, 9, 10, 12, and 14 only were searched